NUTRITIONAL FACTORS AFFECTING MINERAL STATUS AND LONG TERM CARRY-OVER EFFECTS IN RUMINANTS

BY

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Four experiments were conducted with sheep to study the nutritional factors affecting mineral status and long-term carry-over effects on animal performance, blood parameters and mineral composition of selected tissues. In the first experiment, twelve Florida native crossbred lambs were randomly assigned to two treatment groups, where two levels of energy-protein (LEP = .8 x maintenance; HEP = 1.8 x maintenance) and two levels of minerals (LM, approximate requirements; HM = 2 to 30 times the requirements) were studied as they affected Ca, P and Mg retention. Wethers fed HEP + HM or LM diets had greater gains than those receiving LEP diets. Increased dietary levels of energy-protein did not affect serum mineral concentrations. Low mineral-HEP diets apparently depressed liver Fe, Cu and Co. In trial 1 and trial 2, Ca, P and Mg retention was higher in HEP versus LEP diets.

In the second experiment, effects of two levels of energy-protein on sheep mineral status (macro elements) and two levels of minerals on mineral storage and long-term carry-over effects in sheep were studied. Forty-eight Rambouillet crossbred ewe lambs (28.5 kg initial body weight) were randomly assigned to four experimental diets in a 2 x 2 factorial arrangement. Treatments with high minerals were administered for only four months of growing period; ewes were fed LM diets with HEP and LEP levels for the remainder of the trial. Levels of energy-protein were more important than levels of minerals in animal performance. No carry-over effects were observed in hematocrit, Hb, and serum Ca, P, Mg, Na and K. Feeding high minerals resulted in a carry-over effect of bone rib P concentration, expressed as percent of dry, fat-free bone, percent of bone ash and mg/cc until breeding period; but for gestation-parturition period, no carry-over effect of P was observed.

In the third experiment, effects of two levels of energy-protein on mineral status and two levels of minerals on mineral storage and long-term carry-over effects in sheep were studied for trace elements. No carry-over effects were observed in serum Fe, Cu, Zn and Se in ewes fed the original experimental diets (HM) for four months. Carry-over effects were observed in liver Mo concentrations of ewes fed HM + LEP diets until the breeding period and in ewes fed HM + HEP diets until gestation-parturition period. No carry-over effects were observed in liver Fe, Cu, Zn, Mn and Se.

In the fourth experiment, no carry-over effects were found in serum metabolic blood profiles (SMAC 25) of ewes fed HM diets for a four month period.

CHAPTER I
INTRODUCTION

Low ruminant productivity in the tropics is due to many limitations, the most apparent being the low feed intake and poor nutrition (protein, energy, minerals) which consequently result in poor growth and reproductive rates. In areas of the world where beef and dairy cattle enterprises depend on natural or improved grassland for the supply of nutrients, animals are likely to be subjected to undernutrition during the dry period of the year. During those periods, herbage will have a low nutritive value, the most overriding factor being a shortage of digestible energy and protein. Cattle grazing such herbage are unable to meet their requirements for maintenance and generally growth, reproduction and lactation are impaired.

The dietary requirements for minerals are more difficult to accurately define than those for the organic nutrients because many factors determine the utilization of minerals. Starving cattle were observed frequently during the long dry season periods in tropical savannas where native pasture is the only source of feed during the whole year. Low nutritive value of native pastures causes slow growth rates and low reproductive performance. Fluctuation of nutrient content of the pasture results in a particular pattern of growth rate of animals on native grasses, that is, rapid growth in the rainy season followed by a loss of body weight during the dry season. A loss of 30 to 50 kg per animal is not uncommon during a 5- to 6-month dry season. During the dry season, energy and/or protein deficiencies limit cattle production but during

the rainy season, mineral deficiencies may be the major factor which affects rate of cattle production, because protein and digestible energy contents of growing grasses are adequate to meet their requirements for maintenance and production.

Cattle do, however, survive such periods of undernutrition by utilizing their energy conservation mechanism. They are able to mobilize and deplete body tissue reserves during periods of food scarcity and to replenish the reserves when food is readily available. The animals which suffered the greater weight loss tended to lose more body water and protein; during the earlier stages of undernutrition, the tissue lost is almost entirely body fat. Therefore, a multitude of factors influence the productivity of cattle in tropical areas, particularly inadequate nutrition during the long dry period.

The objectives of this research were as follows: 1) To investigate the effect of different energy and protein levels on mineral utilization by the animal; 2) To compare two mineral levels (high and low) on mineral storage and long-term carry-over effects; and 3) To compare two energy-protein dietary levels on mineral status and mineral composition of selected tissues.

CHAPTER 2
LITERATURE REVIEW

Mineral Status of Animals

Minerals play important metabolic roles in animal nutrition; however, they are not a source of energy and protein but are essential for biosynthesis of essential nutrients. Mineral deficiency usually involves several minerals as well as other conditioning factors; however, the deficiency symptoms of one mineral may predominate and affect the performance of the ruminant. The numerous mineral interrelationships in nutrition are not completely understood. In addition, interrelationships of minerals with vitamins, protein and energy further complicate the problem of providing adequate nutrition. Mineral interrelationships also exist between soil, plants and animals.

Numerous mineral deficiencies, imbalances and toxicities are economically important in livestock production throughout the world. For several decades, a major goal in mineral research has been to discover and/or develop simple and accurate biochemical measures of the status of animals for the minerals in which there are important practical problems (Miller and Stake, 1974). Like soils and plants, animal mineral status is influenced by many factors. Nevertheless, when appropriate diagnosis is used, animal tissue concentrations are often better indicators of the mineral status of livestock than either plant or soil concentrations (McDowell, 1976).

Many biochemical measures employed in diagnosing mineral problems involve analysis of tissues and fluids of excreta. The most useful normally follow discovery of fundamental information on the element; cobalt and iodine are excellent examples (Mills and Williams, 1971). Estimation of whole blood or plasma mineral concentrations has wide application (Underwood, 1971). However, often blood measures are employed where they are not especially useful. Because of the essential role of many minerals in enzymes (for example, serum ceruloplasmin and copper), enzyme activity has been developed into a useful measure in certain mineral deficiencies. Perhaps this approach will have far wider application in the future (Mills and Stake, 1974).

At present, 22 mineral elements are believed to be essential for the higher forms of animal life. These minerals comprise seven major or macronutrient elements, calcium (Ca), phosphorus (P), potassium (K), sodium (Na), chlorine (Cl), magnesium (Mg) and sulfur (S), and 15 trace or micronutrient elements, iron (Fe), iodine (I), zinc (Zn), copper (Cu), manganese (Mn), cobalt (Co), molybdenum (Mo), selenium (Se), chromium (Cr), tin (Pb), vanadium (Va), fluorine (F), silicon (Si), nickel (Ni) and arsenic (As). The essentiality of the last six, often referred to as the "newer" trace elements, is based almost exclusively on growth effects with animals in highly specialized conditions. In addition to the 22 essential minerals, all plant and animal tissues contain a further 20 to 30 mineral elements, mostly in small and variable concentrations (Underwood, 1981).

Factors Affecting Mineral Status of Grazing Ruminants

Ideally, animal scientists would like to determine the mineral status of an animal by measuring the mineral content of one tissue which is

readily available from a live animal (Conrad, 1978). However, this is not possible for many reasons. All mineral deficiencies and most excesses, in their more severe forms, are manifested by characteristic clinical and pathological disturbances in the animal. When taken in conjunction with clinical and pathological observations, appropriate chemical analyses and biological assays of tissues and fluids of animals are valuable aids in the early detection of mineral abnormalities in livestock. The choice of tissue or fluid for analysis varies with the mineral under investigation. Blood, urine, saliva and hair have obvious advantages because of their accessibility without sacrifice of the animal. Body tissue sampling presents more difficulties, although simple liver and tail bone biopsy techniques are now available (Underwood, 1981). Tremendous progress has been made during the past 50 years in identifying and correcting these mineral deficiencies and toxicities. However, in the major ruminant livestock-producing areas of the world, relatively little is known about mineral status of soils, vegetation or animals. Mineral analyses are complicated and expensive. Therefore, we are interested in selecting and analyzing the minimum number of tissues which are most indicative of the mineral status of the animal (Conrad, 1978).

Many research approaches have been suggested for detecting mineral deficiencies or imbalances in livestock. According to Underwood (1966), a good diagnosis of mineral disorders in animals should include clinical signs, pathological examinations and chemical analyses of soils, animal tissues and feeds. However, subclinical deficiencies or even acute mineral deficiencies are difficult to diagnose since many deficiencies have a common expression as poor performance in animals. The Netherlands'

Committee on Mineral Nutrition (CMN, 1973) has also indicated that clinical signs alone cannot be used as a guide for identifying a given mineral deficiency unless it is confirmed by tissue analysis as well as feed analysis. The mineral status of the animal is influenced by genetic variation, age, physiological state, level of production, biological availability of the mineral in the diet, parasitism, season and soil ingestion.

Genetic Variation

The effect of breed differences on mineral requirements has often been observed in livestock (Phillips, 1956; Correa, 1957; Wiener and Field, 1969). In Brazil, Bos indicus exhibited clinical Co deficiency when fed forage containing 0.080 ppm Co while Bos taurus were not affected until the Co level dropped to 0.05 ppm or lower (Correa, 1957). Payne (1966) suggests the possibility that unacclimatized cattle of temperature type which sweat profusely and lose saliva and mucus from the mouth may lose significant quantities of minerals, particularly in the arid tropics. There is also extensive evidence for marked animal variation within breeds for the efficiency of absorption of minerals from the diet: 3-35% for Mg in dairy cows, 40-80% for P and 2-10% for Cu in adult sheep (Field, 1981). When different breeds of sheep grazed certain pastures in Scotland, one breed exhibited signs of Cu poisoning whereas another showed signs of Cu deficiency (Wiener, 1966; Wiener et al., 1977). The most probable cause for this apparent breed variation in dietary requirements for some micro-elements could be genetic differences in the efficiency of absorption of the mineral in the diet (Field, 1981).

Enormous differences exist in the extent of practical problems which exist among species and classes of animals for a given mineral element (W. Miller, 1981). For instance, with Zn, the greatest practical problems

are deficiencies in swine feeding. In contrast, swine are virtually never deficient in Mn. Without special attention, Mn deficiency can easily be a serious problem with poultry (W. Miller, 1981). Even the genetic makeup of a given species of animal can materially affect mineral requirements and metabolism. The far higher instance of milk fever among Jerseys than Holsteins reflects such an effect (Miller, 1979). Likewise, Blackface sheep require and tolerate substantially more copper than Welsh (Wiener and Field, 1970).

Wiener et al. (1978) found breed differences in the absorption of dietary Cu by growing lambs and Field and Suttle (1979) found much greater variation in the absorption of Mg between monozygotic twin cows. It is, therefore, suggested that future recommendations of dietary requirements, particularly minerals, will take into account breed differences in the needs of the animal (Field, 1981).

Age Variation

Young animals may be more efficient in metabolizing specific nutrients than mature animals. In mature cows, homeostatic control mechanisms which regulate the Zn content of tissue are much more effective than in calves; therefore, mature cows probably are able to tolerate higher concentrations of dietary Zn (Kincaid et al., 1976). As indicated by Rook and Storry (1962), about 30% of skeletal Mg in young animals can be mobilized under conditions of Mg deprivation while in adult animals, only 2% of bone Mg can be used for physiological needs.

Physiological State and Level of Production

Mineral requirements are highly dependent on the level of productivity and physiological state of the animal (NRC, 1976). As an example, a young, pregnant beef cow during her first lactation would have

substantially higher mineral requirements than a mature dry cow. High-yielding milking cows obviously require much more dietary Ca and P than low-yielding cows because of the richness of milk in those elements. The Ca requirements of laying hens tend to follow a similar pattern with increasing egg production but those of P do not. Growing chicks and pigs consume similar types of diet but chicks require nearly twice the dietary concentration of Ca and some 20 times the concentration of Mn required by growing pigs (Underwood, 1981). The minimum Zn requirements for spermatogenesis and testicular development in young male sheep are significantly higher than they are for body growth (Underwood and Somers, 1969).

Biological Availability

The relative biological availability of the desired element in a compound or supplement is one of the major considerations in the selection of a suitable source of the element (Ammerman and Miller, 1972). Numerous dietary factors, including protein source and level, interrelationships among the mineral ions and certain chelating agents, influence the utilization of mineral ions. With some elements, the chemical form has a major impact on the availability of the element. For instance, Fe is far more available as ferrous sulfate than as ferric oxide (E. Miller, 1981). Likewise, frequently, ferrous compounds are much more available than ferric compounds. Generally, monovalent elements such as Na, K and Cl are highly available. Even so, the effect of the monovalent element, F, is materially affected by the nature of the monovalent element; sodium fluoride is more toxic than the same amount of F in phosphate compounds (Miller, 1981). No element is ever completely absorbed and utilized; some of it is always lost in the normal digestion and metabolic processes. Before a required nutrient can be of nutritional value, it must be in a

form that can be digested, absorbed and transported to the part of the body where it is utilized for its essential function (Peeler, 1972).

Other constituents of the diet often have a major impact on the amount of minerals needed and tolerated. For instance, the Cu requirement and tolerance are very closely related to the Mo in the diet. As the Mo increases, the need and tolerance for Cu also increases. Even the form of the Mo seems to have an influence, with that in natural forages having more impact than added as inorganic Mo (Miller, 1979).

In many respects, the dietary requirements for minerals are more difficult to accurately define than those for the organic nutrients because many factors determine the utilization of minerals. For example, interrelationships among minerals or relationships between minerals and organic fractions may result in enhanced or decreased mineral utilization. Numerous mineral interrelationships which affect requirements and mineral status of the animal include Ca-P, Ca-Zn, Cu-Mo sulfate, Cu-Fe, Se-Ar, Se-S, Fe-P, Na-K and Mg-K. The organic constituents of the diet can have a major impact on the amounts of different mineral elements needed and tolerated. A good illustration is the relationship between vitamin E and Se, and vitamin B_{12} and Co; the effect of vitamin D on Ca and P metabolism is also well known. Goitrogenic substances and chelates such as oxalic acid and phytic acid each influence specific mineral requirements (McDowell, 1976). A good example of these mineral interactions was reported by Suttle and Mills (1966); signs of Cu toxicity in pigs fed 250 ppm Cu or Cu sulfate as a growth stimulant appear when the concomitant dietary intakes of Zn and Fe are "normal"; i.e., adequate in the absence of supplementary Cu sulfate; but no such signs of Cu toxicity appear when additional Fe and Zn are supplied at the rate of 150 ppm Fe

and 150 ppm Zn. In fact, this level of supplementation with Fe and Zn afforded protection against Cu given at the extremely high level of 450 ppm (Underwood, 1979).

In conclusion, many factors, such as age and physiological status of the animal (growth, lactation), levels of various dietary components, duration and route of exposure, and biological availability of the compound, influence the level at which a mineral element causes an adverse effect and consequently affects mineral status of the animal (NRC, 1980).

Other Factors Affecting Mineral Status of Animals

Parasites affect the mineral status of animals; according to McDowell (1976), parasitism can produce Fe deficiency in grazing animals. Strongylus has been shown to reduce liver Cu concentration in ruminants (Boyazoglu, 1973).

Adequate intake of forages by cattle is essential in meeting mineral requirements. Factors which greatly reduce forage intake, such as low protein (< 7.0%) content and increased maturity, lignification and stem-leaf ratios all reduce the total mineral consumed (McDowell, 1976).

Another important factor to take into consideration in tropical areas is the effect of season on the mineral status of the animal, particularly in areas where pasture is the only source of feed for the cattle during the whole year. The mineral composition of forages varies according to many factors; among these are the age of the plant, the soil and fertilization, differences among species and varieties, seasons of the year and the cutting intervals (Gomide, 1978). Seasonal factors like light, temperature and rain could justify certain variations in the chemical composition of forage during the year. The marked decline of P and K as plants mature is not paralleled by comparable declines in trace

minerals. Whole plant concentrations of trace minerals may increase, decrease or show no consistent change with stage of growth, plant species or soil and seasonal conditions. Changes in trace mineral concentrations of forages related to season and stage of growth are of greater significance to the animals in areas in which marginal levels are present (Conrad, 1978). On the basis of low tropical forage mineral concentrations during the dry season, it is logical to assume that cattle would most likely suffer mineral inadequacies during this time. However, grazing cattle were more prone to develop Co or P deficiencies and the clinical signs were most severe after the rains when pastures were green and plentiful. Increased incidences of mineral deficiencies during the wet season are less related to forage mineral concentrations than to the greatly increased requirements for these elements by the grazing animal (Correa, 1957; Van Niekerk, 1974; McDowell, 1976). During the wet season, livestock gain rapidly since energy and protein supplies are adequate. Associated with rapid growth during the wet season, mineral requirements are high while during the dry season, inadequate protein and energy result in the animal losing weight, thereby greatly limiting mineral requirements (McDowell, 1976).

The grazing animal obtains its intake of microelements from a variety of sources: from different plants in a mixed sward, from different parts of the same plant and from the soil (Field, 1981). In view of the importance of soil ingestion as a source of mineral elements to the grazing animal, Healy (1973) suggested not only the usual sequence, soil-plant-animal, be considered but also a direct soil-animal effect. Grazing livestock obtain part of their mineral supply from sources other than forage, particularly from water and soil. Peak soil ingestion is favored

by soils with a weak structure and poor drainage, by high stocking rates, by high earthworm populations and during the dry season when pasture growth is poor (McDowell and Conrad, 1977). In New Zealand, animal ingestion of soil can reach 75 kg for sheep and 600 kg for dairy animals (Healy, 1974). These amounts would represent daily intakes of approximately 200 and 1600 g, respectively, for sheep and cattle. Mayland et al. (1975) determined soil ingestion by cattle under semi-arid conditions in southern Idaho and found values ranging from 3 to 30% of soil in fecal dry matter, with an average of 14%. Soil and dust contamination of herbage can at times provide a further significant source of minerals to grazing animals, especially when grazing intensity is high or when pasture availability is low. With elements such as Co and I which occur in soils in concentrations usually much higher than those of the plants growing on them, soil ingestion can be beneficial to the animal (Underwood, 1981). By contrast, the Cu antagonists, Mo and Zn, are biologically available in soils and their ingestion from soil contamination of herbage may be a factor in the etiology of hypocuprosis in cattle and "swayback" (Cu deficiency) in sheep (Suttle et al., 1975).

Rosa (1980) studied the effect of soil ingestion in sheep and reported that inclusion of 10% Costa Rican soil decreased body weight, increased unabsorbed P and decreased apparent and true P absorption. Also, metabolic fecal P and P retention values were numerically lower. In a recent publication, Fries and Marrow (1982) reported a study of soil ingestion by dairy cattle. Fecal samples were obtained from animals of various management groups from nine dairy herds. Titanium of feces was the indicator of soil ingestion which was calculated for 60% digestibility of the total ration dry matter. The authors found mean soil ingestion

as percent of dry matter intake by groups of yearling heifers and dry cows ranged from .52 ± .11 to .81 ± .10 for those confined to concrete; from .25 ± .04 to 2.41 ± .26 for those with access to unpaved lots with no vegetation; from 1.56 ± .21 to 3.77 ± 1.50 for those with access to unpaved lots with sparse vegetation; and from 1.38 ± .33 to 2.46 ± .50 for those on pasture but receiving supplemental feed as well. The average soil intake by herds on pasture ranged from 4 to 8% of dry matter intake when the cows received no other feed (Healy, 1968). Ingestion of soil by grazing sheep (Healy and Ludwing, 1965) and beef cattle on range (Mayland et al., 1975) was similar relative to dry matter intake to that for dairy cows. Soil ingestion by both cattle and sheep could be reduced markedly when pasture was supplemented by other feeds (Healy, 1968; Healy et al., 1967; Healy and Drew, 1970).

Essential Major Mineral Elements

Calcium and Phosphorus

Metabolism in ruminants

Calcium and P have been recognized as important essential mineral elements. They are major constituents of bones and teeth, and their roles in numerous other physiological and biochemical processes are crucial to the well-being of all animals. Calcium and P depend on vitamin D and Mg for proper utilization. Naturally occurring deficiencies of Ca and P in domestic animals usually develop in quite different circumstances and a dual deficiency, in which the two minerals are equally limiting, is rare. Phosphorus deficiency is predominantly a condition of grazing ruminants, especially cattle, whereas Ca deficiency is more a problem of hand-fed animals, especially pigs and poultry (Underwood, 1981).

Due to highly regulated control mechanisms, most animals will deplete a considerable portion of bone Ca reserves before most other functions are impaired by lack of Ca. In contrast, several functions and performance measures, including growth, reproduction and milk synthesis, can be impaired very quickly by inadequate dietary P (Miller, 1981).

Calcium is required in large quantities for skeletal development and approximately 99.5% of the body Ca is found in the bones. Therefore, Ca requirements are highest during periods of rapid skeletal development and during periods of lactation since milk contains approximately .12% Ca (Conrad, 1978). Although osteogenesis constitutes the major demand for Ca, it also has several regulatory functions.

Phosphorus is a factor in the metabolism of almost all nutrients because of its role in enzyme activity. Approximately 80% of P is found in the osseous tissue where it constitutes about 16.5% of the bone ash. The remaining 20%, found in soft tissue, has vital metabolic roles in carbohydrate metabolism in the formation of hexosephosphate, creatine phosphate and adenylic acid, and in protein metabolism where it is present in nucleoproteins and phosphoproteins (Conrad, 1978).

Normal metabolism of Ca and P requires an adequate supply of vitamin D which is especially involved in intestinal absorption of these minerals. Severe deficiency of Ca and P causes inadequate calcification of the growing skeleton and other deviations in bone development, of which the best known is rickets in young cattle, also often caused by deficiency of vitamin D. In mature cattle, excessive demineralization of the skeleton, called osteoporosis, may result (CMN, 1973).

The most sensitive and earliest biochemical measure of P deficiency is a reduction in serum inorganic P (Underwood, 1966). Values

consistently below 4.5 mg/100 ml in poultry, cattle and sheep or below 6.0 in swine are indicative of P deficiency. However, the CMN (1973) did not consider serum inorganic P to be sufficiently sensitive to recommend it in diagnosing problems with cattle as forage analyses give earlier and more detailed information. Even though serum Ca does decline with deficiency, especially in some species and ages of animals, the homeostatic or physiological mechanisms regulating it are more effective than for P or most other minerals (Underwood, 1966; Miller, 1970).

Concentration of Ca in blood plasma is subject to hormonal control. Normal concentration is 9 to 12 mg/100 ml. Lowered values occur in newly calved, highly productive cows. In cows with milk fever, values fall markedly even if the ration contains sufficient Ca; with rations severely deficient in Ca, deficiency is particularly likely in young cattle (CMN, 1973). Calcium deficiency is not common among grazing cattle, except for high milk-producing cows or those kept on pasture produced by acid and sandy soils of humid areas with no legumes (Underwood, 1966).

Parturient hypocalcemia is a metabolic disease associated with parturition and the initiation of lactation. It is characterized by hypocalcemia, hypophosphatemia and hypomagnesemia. This failure of the Ca homeostatic mechanism has been associated with many factors (Jorgensen, 1974; Hibbs, 1950). Recent theories as to the cause of parturient paresis are related to the metabolism of vitamin D. In normal metabolism, vitamin D is converted to 25-hydroxycholecalciferol (25-OHD) and then to 1,25 $(OH)_2D$ in the kidney prior to exerting its potent Ca mobilization action on bones and intestines (DeLuca, 1974). A failure of the kidneys to biosynthesize sufficient 1,25$(OH)_2D$ was suggested as a possible explanation for parturient hypocalcemia (Fraser and Kodicek, 1970).

However, recent studies have demonstrated that the kidney is responsive to this hypocalcemia (Horst et al., 1977; Horst et al., 1979). It has therefore been proposed that parturient hypocalcemia results from an unexplained target organ resistance to one of the Ca-mobilizing hormones (Yarrington et al., 1977; DeLuca, 1977).

Excessive dietary Ca interferes with the metabolism of several other mineral elements and some organic constituents of the diet. The importance of Ca:P ratios is widely recognized but this ratio is less critical with ruminant animals than in simple stomach animals. Phosphorus contributes about 1% of the total animal body weight but unlike Ca, only 80% of the total quantity is found in the bones and teeth. The remaining 20% is distributed throughout the body in every living cell, being involved in almost all metabolic reactions (Church, 1971). About 10% is combined with protein, lipids and carbohydrates and in other compounds in blood and muscle. The remaining 10% is widely distributed in various chemical compounds (Harper et al., 1979).

Range cattle depend heavily on native pasture for a large share of their P and Ca needs. Most pasture and range forage contains adequate amounts of Ca. Legumes are an excellent source and usually supply the animals' needs when included in the diet (NRC, 1975). In plants, P is an important constituent of a number of biologically important organic compounds such as nucleoproteins, sugar phosphate, ATP, etc., and deficiency symptoms including chlorosis and death of older leaves, greatly reduced plant size and purple coloration due to anthocyanin production (Whiteman, 1980).

Sousa (1978), in northern Mato Grosso, Brazil, reported the highest mean forage P concentrations as .2% during the wet season compared to

.08% P during the dry season. Calcium, however, was higher during the dry season (.67%) than during the wet season (.34%).

Assessment of Ca and P status

Blood plasma. The concentration of Ca in blood plasma is influenced only by severe deficiency whereas that of inorganic P cannot be used as a practical criterion at all (CMN, 1973). Underwood (1966) considered Ca levels in blood plasma as a good indicator of the Ca status of grazing animals and suggested 9-11 mg Ca/100 ml blood plasma as the normal level. Cunha et al. (1964) reported the value of 10-12 mg Ca/100 ml blood serum as the normal level for healthy cattle, with deficiency occurring when these values fall below 8 mg/100 ml. Wise et al. (1963) found that because of the homeostatic mechanism, cattle tended to resist the depletion of plasma Ca. Underwood (1966), on the other hand, indicated that heifers and young cows, having a more efficient mechanism for mobilizing Ca from the bone, were more resistant to milk fever than older cows. In sheep, blood levels of Ca below 9 mg/100 ml serum indicate a Ca deficiency (hypocalcemia) according to the NRC (1975). Lebdosoekojo (1977) reported that serum Ca levels were not affected by complete mineral supplementation but seasonal fluctuations were noted.

Duncan (1958) reported that P was less readily mobilized from bone than Ca. Thus low serum inorganic P concentration is the first indication of a dietary P deficiency. Cunha et al. (1964) considered cows with concentrations lower than 5 mg P/100 ml serum as deficient. Similarly, Underwood (1966) indicated that plasma inorganic P was a satisfactory criterion for assessing the P status of animals. Underwood (1966) reported that in P-deficient animals, there is a negative correlation between plasma P and plasma Ca. As plasma P decreases during P deficiency,

plasma Ca increases until values of 13 to 14 mg% are reached. Chicco et al. (1973) reported that dietary P supplementation increased plasma P levels but depressed plasma Ca concentration; conversely, high dietary Ca tended to reduce plasma P levels. Reed et al. (1974) reported that serum inorganic P levels were increased by supplementing cattle with bone meal but Ca and Mg levels were depressed.

Inorganic P contents of plasma differed between animals in a herd and between months within animals (Blosser et al., 1951). There was a tendency for inorganic P concentration in plasma to decrease just prior to parturition as did Ca concentration, with the lowest point at the time of calving (Wilson et al., 1977). McAdam and O'Dell (1982) studied mineral profiles of blood plasma of lactating dairy cows and found that at parturition, plasma P concentration decreased for all animals except young cows fed plain salt. Phosphorus concentration remained fairly constant for animals on each treatment (salt versus minerals) throughout lactation, with only minor elevations and depressions.

Nursing cows grazing P-deficient pasture in Florida had an average serum P value of 2.55 mg/100 ml of serum (Becker et al., 1933). This P level was raised to 4.02% after the cows had been supplemented with bone meal. Increasing inorganic P in the plasma results in the formation of a colloidal Ca-phosphate complex that is rapidly removed from the circulation (Irving, 1973). Kitchenham et al. (1975) found the mean blood serum inorganic P of the rapidly growing calves to be higher ($P < .01$) than in conventionally reared heifer calves. Growth rate was significantly correlated ($P < .05$) with concentration of serum inorganic P in calves reared by the conventional system but not with the rapid growth system. Plasma percentages of inorganic P were highest in first

lactation cows and declined with subsequent lactations. Season of calving had a significant effect on plasma inorganic P, with cows calving in November to December having the highest concentrations. The season of calving effect on plasma inorganic P cannot be attributed to diet since ration components and proportion of grain to concentrate remained constant during the trial. Also, time of sampling should be standardized to reduce the effect of diurnal variation in plasma concentration of inorganic P (Forar et al., 1982).

The CMN (1973) considers that the concentration of inorganic P in blood plasma varies widely because of factors not well understood. Little et al. (1971) reported that the rise in inorganic P of blood plasma, which occurs with time, is due to hydrolysis of organically combined P; in this case, ATP from the cellular fraction. Inorganic P in blood is markedly affected by recent dietary P intake in cattle (Little, 1968, cited by Little, 1972). Another experiment conducted by Cohen (1974) reported that blood plasma P concentration in cattle was significantly related to P intake ($P < .05$) but the relationship varied ($P < .05$) depending on time of the day at which samples were collected. In the work done by Gartner et al. (1965, cited by Little et al., 1971), the blood plasma inorganic P was substantially increased by excitement and exercise.

Levels of P in blood plasma of cattle from two different regions of Costa Rica were reported by Kiatoko (1976) to be $3.3 \pm .6$ and $3.2 \pm .5$ mg/100 ml of plasma. The same author studied the mineral status of beef cattle herds from four soil order regions of Florida and found that mean plasma P in all regions was above the critical level of 4.5 mg/100 ml reported by Underwood (1966) during the wet season, while during the dry season, concentrations in the southeast region (Histosol soil order) were

below this level. Of all the animals studied, 13% had low plasma P during the dry season (Kiatoko et al., 1982).

Lebdosoekojo et al. (1980) studied the mineral nutrition of beef cattle grazing native pastures on the eastern plains of Colombia and reported the concentrations of serum minerals in cows given supplemental NaCl; only serum P was below the deficient level during the rainy season but during the dry season, P content of the forage increased. However, forage intake was low because of limited availability. Therefore, an increase in serum P concentration was apparently due to a decrease in P demand at the tissue level or an increase in availability of P in the forage.

Bone Ca and P. About 99% of the Ca and 80% of the P are found in the bones and teeth so that bone formation and maintenance are quantitatively their most important functions (Underwood, 1981). Abnormalities of bones and teeth can occur at any age. Rickets is the term used to denote the skeletal changes which result from defective calcification of the growing bone in young animals. Osteomalacia is used to describe the condition in which excessive mobilization of minerals, particularly Ca and P, has occurred in adult bone. Withdrawal of Ca and P from the bones occurs normally and regularly in dairy cows at the height of lactation and in hens during intensive egg-laying, even when intakes are otherwise adequate. Withdrawal of minerals during periods of inadequate intake does not take place equally from different parts of the skeleton. The spongy bones, ribs, vertebrae and sternum, which are the lowest in ash, are the first to be affected. The compact shafts of the long bones such as humerus, femur and tibia and of the small bones of the extremities are the last reserves to be used. In each case, the essential change is a

reduction in the total mineral content of the bones, with little alteration in the proportions of the minerals in the remaining ash (Underwood, 1981). In a longer experiment (14-18 months) with growing sheep fed a moderately P-deficient but otherwise adequate diet, the total ash concentration of the ribs and vertebrae were some 20% lower and that of the long bones over 8% lower than those of similar bones from sheep on the same diet supplemented with phosphate (Stewart, 1934-35, cited by Underwood, 1981).

Bone ash consists almost entirely of Ca and P salts and the relative amounts of these elements show little variation; consequently, the ash content of bone is commonly used as a measure of the state of Ca and P nutrition (Maynard and Loosli, 1971). The nature of the diet can affect the mineral relationships in bone, even though the ash content is not appreciably changed. In mammals, the bone is made of approximately 36% Ca, 17% P and .8% Mg, based on dry fat-free bone, as reported by Maynard and Loosli (1971). Bone is not static structure; there is an active metabolism. Isotope studies have shown that there is a continuous interchange of Ca and P between the blood and bone and between various parts of the bones. Therefore, Ca and P in the body are in dynamic state, similar to the situation for fat and protein, and the net result of the interchange determines the nutritional status with respect to a given physiological need (Maynard et al., 1979).

Little (1972), in Australia, reported that cattle fed a P-deficient ration (8% crude protein and .08% P, dry matter basis) for 6 weeks showed levels of 66.8 ± 2.7 and 61.8 ± 1.5% rib ash, 24.5 ± .5 and 23.8 ± 1.7% Ca and 11.5 ± .5 and 11.1 ± .4% P on dry fat-free bone basis at the beginning and end of this period, respectively.

Ammerman et al. (1974) conducted an investigation of mineral composition of tissues from beef cattle under grazing conditions in Panamá and reported levels of 60.5 to 67.7% for bone ash, 37.6 to 38.2% for Ca and 17.6 to 18.1% for P. Kirk et al. (1970) reported that cows on phosphate fertilized pastures had metacarpal and metatarsal bones of greater density than cows on unfertilized pastures; the respective values were 2.00 to 2.05 g/cm^3 as compared to 1.96 g/cm^3. Kiatoko (1976) obtained a value of 48.2 to 55.6% bone ash on dry, fat-free bone basis in cattle grazing on Costa Rican farms which had less than .33% P in the forages. Mendes (1977) found bone ash Ca means ranged from 36.96 to 38.45% and P ash from 15.13 to 15.54%. When he expressed these means as percent dry, fat-free bone, the Ca means ranged from 22.7 to 24.8% while P ranged from 9.38 to 10.1% in cattle.

Cohen (1973) correlated the P concentration in pasture, blood, hair and bone samples. There was a significant correlation ($r = .97$) between P in the pasture and in the dry, fat-free ribs. Bone density, bone ash and mineral concentrations of whole metacarpal bones were lower during the rainy season for bulls receiving NaCl only (Lebdosoekojo et al., 1980). This finding suggests that the animals had mobilized bone reserves during the rainy season to sustain body needs and it is in agreement with the serum data.

Pond et al. (1975) reported that pigs fed high Ca:P ratios (1.2:1) had a higher bone ash content than the pigs fed a normal Ca:P ratio (.5:.4) in the diet. In another experiment conducted with pigs, Bayley et al. (1975) reported bone ash and breaking strength were increased by P supplementation at levels of .32 and .48%. As reported by Benzie et al. (1959) ribs of ewes were more readily rehabilitated and resorbed

than long bones; consequently, status of certain minerals is better detected by ribs. However, Blincoe et al. (1973) found no difference in Ca and Mg concentrations in caudal vertebrae, right rib and femur. Campo and Tourtellote (1967) reported Ca and P contents in spongy parts of long bones of calves of 36.5 to 37.1% and 16.4 to 18.7%, respectively.

Sousa et al. (1979) reported mineral data from six farms in northern Mato Grosso, Brazil. Rib bone ash means were 37.7 ± 2.5 and 15.5 ± .06% for Ca and P, respectively, during the dry season and 37.6 ± 2.7 and 15.0 ± .7% for Ca and P, respectively, during the wet season. The levels of rib bone ash Ca are considered normal but P is below the normal level and considered as borderline to deficient. Rib bone ash P levels in this study were based on non-productive animals. In the same study, they observed that mean rib ash P during the dry season was higher than during the wet season, even when forage P levels were low, suggesting a higher requirement during the wet season compared with the dry season.

An experiment conducted by Peducassé (1982) in tropical areas of Bolivia to determine the mineral status of beef cattle during the dry season found deficient levels of Ca (< 37.6%) and P (< 17.6%) in bones (as % bone ash). Ninety-six percent of the bone samples had Ca levels lower than 34% bone ash and 45% of the samples were less than 17% P.

Rosa (1980) studied bone ash concentration as affected by P levels in the diet and found that added dietary P produced a 3.9% increase ($P < .01$) in bone ash. Conversely, excess dietary Al affected bone ash by causing a 2.3% reduction ($P < .01$) in that parameter. Phosphorus content in bone ash was also affected by dietary P, with additional P increasing ($P < .01$) bone P from 16.8 to 17.2%. He also reported no major

effects of dietary P, Fe or Al on bone Ca. Valdivia (1977) also observed decreased bone ash percentages in sheep fed 2,000 ppm dietary Al. Recent studies by Hidiroglou et al. (1982) in relation to the chemical composition of sheep bones as influenced by Mo supplementation reported greater concentration in the latter ossification portion of the bone in wethers was unaffected by dietary Mo. The compact shaft contained more ash, Ca, P and Mg than the proximal and distal parts of the bone.

Some research groups consider serum inorganic P to be sufficiently sensitive and recommends it for diagnosing P deficiency. However, it is significant that the CMN (1973) recommends forage analyses because studies have shown that earlier and more detailed information can be obtained. It is apparent that both serum inorganic P and forage P analyses are effective in determining the P status of grazing ruminants and that either or both can be used (Conrad, 1978).

Magnesium

Metabolism in ruminants

The most important practical Mg deficiency problem in farm animals is the condition, largely confined to lactating cows, known as grass tetany (Miller et al., 1974; Underwood, 1966). The clinical signs of tetany are caused by inadequate Mg in serum and other extracellular fluids; however, often low serum Mg does not result in tetany.

Magnesium is intimately associated with Ca and P through distribution and metabolism. Magnesium is the fourth most abundant cation in the body. All tissues of the higher animals contain Mg, usually in considerable quantities; Na, K and Ca are also major constituents. The occurrence of Mg can only be understood when these four major electrolytes are considered together. Approximately 70% of the body Mg supply is in

the skeleton, the remainder being found widely distributed in the various fluids and other soft tissues. One-third of the supply in the bones is subject to mobilization for soft tissue when the intake is inadequate. As indicated by Rook and Storry (1962), about 30% of skeletal Mg in young animals can be mobilized under conditions of Mg deprivation while in adult animals, only 2% of bone Mg can be used for physiological needs. Cardiac muscle, skeletal muscle and neural tissue depend on a proper balance between Ca and Mg ions.

According to Walker and Vallee (1964), Mg takes part in about eighty enzymatic reactions. Magnesium is an active component of several enzyme systems in which thiamine pyrophosphate (TPP) is a co-factor. Oxidative phosphorylation is greatly reduced in the absence of Mg. It is also an essential activator for the phosphate-transferring enzymes myokinase, diphosphopyridine nucleotide kinase and creatine kinase. It also activates pyruvic acid carboxylase, pyruvic acid oxidase and the condensing enzymes for the reactions in the Krebs cycle (Swenson, 1970). Magnesium is vitally involved in the metabolism of carbohydrates and lipids as a catalyst of a wide array of enzymes which require these elements for optimum activity (Walker, 1969). In the light of these functions, it is not surprising that Mg deficiency in animals is manifested clinically in a wide range of disorders, which include retarded growth, hyperirritability and tetany, peripheral vasodilation, anorexia, muscular incoordination and convulsions (Underwood, 1981).

Although the adult cow contains about 250 g Mg, hardly any of it can be released into circulation when intake is insufficient. In contrast, young cattle can mobilize Mg more efficiently from body stores and develop clinical signs only after a longer interval. When Mg supply

and inevitable metabolic losses are in balance and supply is thus just adequate, about 2.5 g Mg is excreted in urine daily. When the supply is more than adequate, excess is excreted in urine and the concentration in blood plasma remains in the normal range. When the amount of Mg absorbed is inadequate, daily excretion in urine drops sharply, sometimes to less than 0.1 g (CMN, 1973).

In ruminants, a physiological Mg deficiency frequently occurs in adult animals when turned into fresh pasture during spring and autumn in certain areas of the world. The pastures on which the animals develop the grass tetany are not usually low in Mg; therefore, there must be other factors related to Mg utilization in ruminants: 1) High K in the herbage is perhaps the most frequent factor implicated in grass tetany. Experiments concerning the effects of K fertilization of pastures on the incidence of hypomagnesemic tetany have yielded inconclusive results (Bartlett et al., 1954; Sims and Crookshank, 1956; Ritchie and Hemingway, 1963). Fontenot et al. (1960) found that high K, high protein diets typical in this respect of lush, young grass depressed Mg retention in sheep. In another experiment, Newton et al. (1972) found that feeding a high K ration (4.9% K) to lambs resulted in a 46% decrease in apparent Mg absorption. Metson et al. (1966), in New Zealand, reported that K depresses the uptake of Mg, Na and Ca. 2) High N fertilization of forages has been shown to increase crude protein content of the forage and has adversely affected Mg utilization in cattle grazing the herbage. Metson et al. (1966) studied hypomagnesemic tetany in New Zealand and found that high protein content of grass is a causal factor in the development of this disease. Head and Rook (1955) postulated that high ruminal ammonia N levels might result in the formation of a complex

between N and Mg and interfere with Mg absorption. Rosero (1975) studied the effects of species, stage of maturity and level of N fertilization on the Mg availability for ruminants and found that Mg retention was lower ($P < .05$) with early stages of maturity (high N content). Also, fertilization lowered the Mg intake, % absorption ($P < .10$) and Mg balance ($P < .05$). 3) Excesses of citric acid on trans-aconitic acid apparently depress blood Mg enough to cause tetany when cattle are under stress. Scotto et al. (1971) indicated that administration by drenching of KCl and citrate or transaconitase would produce a tetany in a high percentage of experimental cattle. 4) Low soluble carbohydrates, low Ca, high P and low Na in the pastures are involved in the metabolism of Mg (Matsen et al., 1976; House and Mayland, 1976). 5) Changes in amounts of soluble Al may also be an important route through which climatic and agronomic factors alter incidence of grass tetany (Allen et al., 1980). Serum Mg levels in Al-treated steers (4,000 ppm Al) dropped within 24 hours after treatment began and declined 32% by the end of 4 days ($P < .01$). After treatments were discontinued, serum Mg levels returned to normal. Also, the authors found that the rumen contents from tetanous animals averaged 2373 ppm Al, more than 5 times the level found in normal fistulated animals.

Assessment of Mg status

Blood plasma. According to the CMN (1973), the approximate normal level of serum Mg in cattle is 2.0 to 3.5 mg/100 ml. Below the level of 2.0 mg Mg, deficiency begins, with 1.0 mg considered an extreme deficiency. However, Cunha et al. (1964) suggested 2.5 mg of Mg/100 ml of serum as a normal level in cattle and reported that calves showing clinical signs of tetany contained levels as low as .1 mg Mg/100 ml serum. On

the other hand, Underwood (1966) considered the normal level of serum Mg in cattle to be 1.8 to 3.2 mg/100 ml.

Kiatoko (1976) reported blood plasma Mg levels of 1.8 ± .2 and 1.9 ± .3 mg/100 ml in cattle from two different regions of Costa Rica. Claypool (1976) studied the factors affecting Ca, P and Mg status of dairy cattle on the Oregon coast and found a large source of variation in plasma Mg was due to herd difference (35%), with means of 2.2 to 2.6 mg/100 ml of serum.

O'Kelley and Fontenot (1969) reported that levels of .18, .19 and .16% Mg in the rations of lactating beef cattle on a dry matter basis in the first, second and third phases of lactation are necessary to maintain a level of 2 mg/100 ml serum Mg. Likewise, levels of .12, .10 and .13% Mg on a dry matter basis for beef cows at 145, 200 and 255 days of gestation, respectively, were needed to maintain that level of 2 mg/100 ml serum Mg (O'Kelley and Fontenot, 1973). Jerez (1982) studied the mineral status of grazing beef cattle in the eastern region of the Dominican Republic, consisting of Romana Red, Criollo and Brahman breeds. Mean serum Mg concentrations for regions 1, 2 and 3 were 2.1, 2.3 and 2.7 mg/100 ml, respectively. Of the 73 serum samples analyzed for Mg, only 19% exhibited concentrations below the critical level of 2 mg/100 ml (McDowell and Conrad, 1977). Most forage concentrations (67%) were adequate in Mg. Kemp (1960) reported a positive correlation between the Mn content in forage and serum. Chicco et al. (1973) reported that high dietary Ca depressed Mg content in bone and plasma and decreased Mg utilization. Conversely, high Mg in the diet reduced plasma Ca. Dietary P had little effect on Mg utilization. A positive correlation was found between dietary Mg and either plasma or bone Mg.

Bone ash Mg. Harrington (1975) found a significant negative correlation between bone ash concentration of Mg (rib, metacarpal) and the length of time foals were fed a Mg-deficient diet. The normal Mg concentrations in rib bone of cattle range from .67 to .70% (Blaxter and Sharman, 1955). Magnesium levels of .19 to .35% rib ash from cattle with hypomagnesemia were reported. Loaiza (1968) reported levels of Mg in metacarpal bone ash from grazing cattle from .56 to .61%.

Mendes (1977) reported that percent bone Mg calculated on dry, fat-free bone basis in the rainy season from six ranches in the northern part of Mato Grosso, Brazil, varied from .46 to .49%. Lebdosoekojo (1977) reported Mg levels of .60 and .73% in rib ash during the rainy and dry seasons, respectively, when bulls were kept on native pasture with only salt as a supplement. For those receiving a complete mineral supplement, Mg levels were .71 to .69% for the rainy and dry seasons, respectively. Sousa (1978), in Brazil, reported rib bone Mg levels from all six farms studied were slightly below normal levels but they were higher than in animals with grass tetany. Rib bone ash Mg levels during the wet and dry seasons were .46 and .49%, respectively. The lower level of rib bone ash Mg during the wet season is probably due to higher requirements when animals are gaining weight rapidly, lactating and performing other production functions.

Urinary excretion of Mg. According to the CMN (1973), daily urinary excretion is a better criterion of Mg supply than plasma concentration. Even Mg concentration in a random sample of urine at any time of day gives a good indication of supply. Tentative criteria are as follows: more than 100 mg/l, adequate to liberal; 20-100 mg/l, inadequate; and less than 20 mg/l, severely deficient, danger of tetany. Within the

usual practical range, the percentage of Mg absorbed is not materially affected by the amount consumed (Miller et al., 1974). Likewise, generally Mg content of tissues is not elevated with excess intake. Rather, homeostasis is maintained by the excretion of excess Mg via urine; thus urinary excretion of Mg is a threshold phenomenon. Substantially more than a trace of Mg in urine indicates adequate absorbed Mg to meet the animal's needs. On the other hand, very low urine Mg indicates either a deficient or only barely adequate intake (Miller and Stake, 1974).

Magnesium conservation by the kidneys is believed to be mediated in part by the parathyroid hormone (PTH). Losses of kidney function and responsiveness to parathyroid hormone in cows consuming diets high in K and organic acids could quickly lead to a mineral imbalance (Deetz et al., 1981a). Magnesium excretion is believed to be mediated by glomerular filtration and tubular resorption. Organic acids such as citric acid may interfere with Mg and Ca resorption by chelating the ions, thus forming complexes that would be poorly resorbed by the renal tubule (Deetz et al., 1981b).

Sodium, Potassium and Chloride

Metabolism in ruminants

Sodium, K and Cl are of great importance in the metabolic processes of all animals, with deficiencies causing rapid impairment of growth and/or production. Sodium and Cl, along with K, function in maintaining osmotic pressure, regulating acid-base equilibrium and controlling water metabolism in body tissues. It is convenient to consider Na, K and Cl together because of the broad similarities in their functions and requirements in the animal body and their interactions with each other, and because Na and Cl are associated in the form of common salt (Underwood, 1981).

Animals need to receive a regular supply of NaCl because there is limited body storage capacity and any excess consumed is rapidly excreted in the urine. When the animal is deprived of NaCl, it is able to conserve the limited body reserves by largely eliminating urinary losses. Even after prolonged severe deficiency, neither blood levels of NaCl nor the amounts secreted in the milk decrease. Thus, lactating animals suffer most from the lack of salt in the diet (Loosli, 1978). Sodium constitutes about .2% of the body and occurs primarily in the extracellular fluids, playing an important function in the regulation of neutrality in the blood (Maynard and Loosli, 1971). It not only functions in the regulation of the balance of body fluids but also plays major roles in nerve impulse transmission, the rhythmic maintenance of heart action and in metabolism of carbohydrates and proteins (Fenner, 1980). On the other hand, Cl is found both in intra- and extracellular fluids, making up two-thirds of the acid ions in the body (Maynard and Loosli, 1971). Thus, it also serves an important function in the maintenance of acid-base balance in the body in addition to being a constituent of HCl in the gastric juice secreted into the stomach. The normal routes of Na excretion are the urine, sweat and feces. Sodium losses from the bowel are usually very low while those from sweat are higher in hot, tropical climates (Pearson and Wolzak, 1982). Urinary excretion is the most important mechanism of Na elimination and is determined by the balance between Na filtered from blood in the glomerulus and that actively reabsorbed from the renal tubules (Pearson and Wolzak, 1982).

The total salt requirement of growing lambs approximates .40% of dietary dry matter. The Na requirement is .04 to .10% of dietary dry matter (NRC, 1975). The requirement for Cl is unknown. Range operators

commonly provide 220-340 g of salt per ewe per month. Some drylot tests show that lambs consume about 9 g daily; mature sheep in drylot may consume more.

The metabolism of NaCl is altered in hypertensive patients. One of the major changes observed is an expansion of the extracellular fluid volume and, in particular, an increase in the blood volume (Pearson and Wolzak, 1982). The renin-argiotensin-aldosterone (RAA) system is known to adjust distal tubular Na reabsorption in the kidney and hence excretion to balance the Na needs of the body (Collings and Spangenberg, 1980, cited by Pearson and Wolzak, 1982). Kolata (1981) found that high extracellular Na^+ ion concentrations have been noted in a variety of cell types from humans and animals suffering from hypertension.

Potassium is the third most abundant element in the animal body, surpassed only by Ca and P. In contrast to Na, which is the main electrolyte in the plasma and extra-cellular fluids, K is present primarily inside the cells. The blood cells, or erythrocytes, contain approximately 25 times as much K as is present in the plasma. Muscle and nerve cells are also very high in K, containing over 20 times as much as that present in the interstitial fluids (Thompson, 1978). Over two-thirds of the body K is found in the muscle and skin. A dressed carcass will contain about 75% of the body K.

Potassium requirements of beef cattle have been reported to be .6 to .8% of dietary dry matter (NRC, 1976). Requirements at this level would be amply met with high forage diets which usually contain several times the amount present in high grain diets. Gomide et al. (1969) studied the mineral composition of six tropical grasses and noted that the average K content at 4 weeks was 1.42% vs .30% at 36 weeks of age.

These workers noted that a low forage K content may be a critical factor in the poor performance of cattle, particularly during the dry season. Loosli (1978) reported that Na deficiency is most likely to occur in animals grazing pastures heavily fertilized with K which depresses Na uptake by grasses. He added that since Na secretion into milk is high even during Na deficiency, lactating animals suffer from lack of salt in the diet.

Assessment of Na and K status

Because of its rapid reaction to deficiency long before clinical signs appear, the best criterion is concentration of Na and K in saliva. Sodium deficiency causes a fall in Na and a rise in K. Normal values in saliva are 3.3 ± .3 Na and .3 ± .1 g/liter K. Less than 1 g/liter for Na and more than 2.5 g/liter for K signal marked deficiencies, sometimes even clinical signs (CMN, 1973). According to Kemp (1964, 1966), if less than 3 g/liter Na is excreted per day, a dietary deficiency is implied. Normal saliva values are given in Meq/liter as 145 for Na and 7 for K, with a Na:K ratio of 20. The comparable values for the deficient animals were 40 to 90, giving a Na:K ratio of .45. This adaptive change in the Na:K ratio of parotid saliva is sufficiently sensitive to have been used to estimate the Na requirements of lactating ewes (Morris and Peterson, 1975, cited by Underwood, 1981).

Due to large reserves of mobilizable body Na, plasma Na is not a sensitive indicator of Na status. Fecal excretion of Na is variable (Kemp, 1964) and not a reliable index of Na status. Milk Na is only slightly decreased with low Na intake. Depressed plasma K is characteristic in K deficiency. Reduced milk K and increased hematocrit readings are observed during K depletion in lactating dairy cows (Pradhan and Hemken, 1968).

Essential Trace Mineral Elements

Iron

Metabolism in ruminants

For many years, nutritional interest in Fe was focused on its role in hemoglobin formation and oxygen transport. Iron is important in electron transport mechanism in cells and as a component of several heme enzyme systems (Church and Pond, 1975). Iron deficiency is one of the most commonly occurring deficiency diseases of swine and humans. It is rarely of practical concern in cattle, sheep and poultry except where precipitated by chronic blood loss due to other conditions. Approximately 25% of total body Fe is stored as ferritin and hemosiderin in the liver, spleen and other tissues (Thomas, 1970). According to McDowell et al. (1978), Fe deficiency is unlikely to occur in ruminants except in circumstances involving blood loss such as parasitic infestation or disease.

The great majority of Fe is contained in hemoglobin located in the erythrocytes. Hemoglobin composes approximately one-third of the red blood cell mass or about 1% of the body weight of man and domestic animals. There is also a considerable amount of Fe stored as ferritin and hemosiderin in the liver and spleen. Hemoglobin contains about .34% Fe but ferritin may contain up to 20% Fe and hemosiderin up to 35% (E. Miller, 1981). There is much less Fe in myoglobin, which is similar to hemoglobin in composition and has much the same function in muscle as that of hemoglobin in the erythrocyte. While transport Fe (transferrin) and Fe enzymes contain a very small percentage of the total body Fe, these forms of Fe are vital. In all species, Fe deficiency results in a hypochronic, microcytic anemia with low serum Fe, increased total serum Fe

binding capacity (TIBC) and a decreased transferrin saturation (Underwood, 1971). In humans, 35-40% saturation is normal (Bothwell and Finch, 1962). Less than 18% transferrin saturation indicates impaired erythropoiesis. Iron deficiency anemia develops in the young domestic animal during the suckling period because of the low level of Fe contained in milk. It develops more rapidly in suckling pigs than in the lamb or calf because of the much greater growth rate of the pig during the suckling period (E. Miller, 1981).

Assessment of Fe status

Ferritin is the main storage compound of the body and its concentration in the tissues, together with that of hemosiderin, reflects the Fe status of the animal. A high positive correlation between serum ferritin concentrations and body Fe stores exists in man (Walters et al., 1973, cited by Underwood, 1981) so that its estimation is a useful diagnostic tool. The principal carrier of Fe in the blood is another non-heme protein compound, transferrin or siderophilin, which is present in the blood of all vertebrate species. Serum Fe and Fe binding capacity, the basis for calculating % transferrin saturation, can be determined simultaneously and by a completely automated procedure (Friedman and Cheek, 1971, cited by Miller and Stake, 1974). However, the limited number of determinations reported for livestock and unknown range of normal variations restrict the interpretation which can be made for this biochemical means of diagnosing Fe deficiency. The body has a limited ability to excrete Fe. Most of the Fe eliminated from the body is via the feces, but this consists primarily of unabsorbed food Fe. Losses from urine and sweat are minor. Even the loss of Fe in milk by high-producing dairy cows is not great because of the low level of Fe in milk, .5 ppm (E. Miller, 1981).

Extensive research on the biological availability of various Fe sources has been conducted in recent years (Ammerman and Miller, 1972). The availability of Fe in different compounds varies enormously (Miller, 1979). Generally, Fe in soluble compounds such as ferrous sulfate and ferric citrate is much more available to cattle than in ferric oxide or Fe phytate (Ammerman et al., 1967; Bremner and Dalgarno, 1973). Levels of dietary Fe exceeding 1000 ppm for growing cattle have generally reduced growth rate and plasma inorganic P levels (E. Miller, 1981).

Liver Fe. The liver is the center of mineral metabolism in the animal body and is a useful organ for estimating the Fe, Cu, Zn, Mn and Co status of animals (Boyazoglu et al., 1972). Hartley et al. (1959) reported that the approximate normal level of Fe in cattle liver ranges from 180 to 340 ppm on a dry matter basis. Cunha et al. (1964) indicated 200 to 300 ppm as the normal level of Fe in the cattle liver. Ammerman (1970) found liver levels of 100 to 300 ppm Fe in cattle in Florida in an adequate state of Cu nutrition; the animals did not show deficiency signs consistently until the Cu levels decreased to 25 ppm.

Among the organs and tissues of the body, the liver and spleem usually carry the highest Fe concentrations, followed by the kidney, heart, skeletal muscle, pancreas and brain. Variation among species can be very high in the liver, kidney and spleen. In some species, notably the rat, rabbit, sheep and man, but not in the dog, the liver has a high storage capacity of Fe (Underwood, 1966). Lebdosoekojo (1977) found that the Fe levels in the liver of young bulls on native pasture with and without complete mineral supplementation were 351 and 408 ppm dry matter, respectively. Watson et al. (1973) found a decrease in liver Fe from 1055 to 678 ppm ($P < .05$) when dietary Mn was increased from 30 to 4030 ppm

in diets for wether lambs. In the liver, the decrease in Fe concentration from 178 to 99 ppm was associated with an increase in the concentration of Cu from 622 to 2521 ppm. Rosa (1980) studied Cu, Zn and Fe interrelationships in sheep and found high dietary Fe produced an elevation ($P < .01$) in Fe accumulation in liver (212 to 788 ppm). High dietary Zn antagonized this effect ($P < .05$) by reducing Fe storage resulting from excess dietary Fe. Standish et al. (1971) reported a significant Fe x P interaction effect in liver Fe values. The authors found that greater intake of P did not significantly affect liver Fe when added to the low Fe diet but with the high Fe diet, it reduced ($P < .05$) liver Fe.

Boyazoglu (1973) and Cunha et al. (1964) observed an inverse relationship between Cu and Fe in the liver. Ott et al. (1966c) observed increased liver Fe in lambs fed a diet containing high levels of Zn. Heinrich (1971, cited by Miller and Stake, 1974) proposed that Fe depots can be determined by measuring "diffuse storage Fe" in the cytoplasm of the bone marrow reticuloendothelial cells. The procedure detects prelatent Fe deficiency but is unsuited for routine use in either man or animal.

Coleman and Matrone (1969, cited by Davis, 1980) presented evidence that in high Zn-fed rats, the amount of ferritin was about one-third that found in rats fed a normal Zn diet. The excess dietary Zn resulted in the formation of an "Fe-poor ferritin." Zinc toxicity did not appear to interfere with the incorporation of amino acids into the ferritin. There was some suggestion that the turnover rate of ferritin Fe and ferritin protein in the Zn-fed rats may have been faster than in rats fed the control diet (Davis, 1980).

Many consider percent saturation to be the most practical means of detecting Fe deficiency in its early states (Underwood, 1971). The Fe content of transferrin reflects available circulating Fe at the hemoglobin synthesis site.

Copper, Molybdenum and Sulfur Metabolism in ruminants

There are advantages in considering Cu, Mo and S together because of their nutritional interrelationships and their profound metabolic interactions.

In many areas of the world, Cu deficiency is a major problem for grazing cattle (Underwood, 1981; CMN, 1973). Except for milk-fed animals, most naturally occurring Cu deficiencies are conditioned by dietary factors (high Mo, sulfide, sulfate or sulfur-containing amino acids) that interfere with Cu utilization. Young and growing animals have a higher Cu requirement and therefore a higher deficiency incidence than mature ones. Young sheep are very susceptible to Cu deficiency (Underwood, 1966), with important genetic differences among breeds (Wiener and Field, 1970). Copper deficiency in livestock results in a wide variety of disorders. In addition to depressed growth, common signs include anemia, osteoporosis, cardiovascular connective tissue defects and demyelination of the central nervous system.

In some areas, the deficiency of Cu results from vegetation low in Cu, but in many locations, it may result from one of several factors in the forage; e.g., the presence of high concentrations of Mo in the forage is one of the predominant factors interfering with Cu metabolism. Forages grown on organic soils of Florida, for example, are high in Mo and their consumption can result in molybdenosis or hypocuprosis in grazing cattle (Becker et al., 1965). Studies of the nutritional physiology

of Cu were given a further impetus by Australian investigations of chronic Cu poisoning in sheep. Copper retention was shown to be dependent upon the Mo status of the diet, and the limiting effect of Mo was shown, in turn, to depend upon the inorganic sulfate status of the diet and of the animal (Dick, 1956, cited by Underwood, 1971). In relation to the distribution of Cu in body tissues and fluids, the adult human body was calculated to contain 110--120 mg of total Cu. Newborn and very young animals are normally much richer in Cu per unit of body weight than adults of the same species. The newborn levels are largely maintained throughout the suckling period, followed by a steady fall during growth from weaning when adult levels are reached (Underwood, 1971).

The fact that cytochrome oxidase contains Cu immediately establishes an essential metabolic role for this microelement. Field conditions actually exist in which sheep grazing pastures of the same or comparable levels of herbage Cu suffer from either Cu deficiency or chronic Cu poisoning. There is a three-way interaction between Cu-Mo and inorganic sulfate, with the primary site in the gut: reduction in the rumen of sulfate to sulfide, reaction of this sulfide to form thiomolybdate, and reaction of the thiomolybdate-$CuMoSO_4$ (Dick et al., 1975, and Suttle, 1975, cited by Underwood, 1979). Adverse effects of Mo on Cu utilization in the tissues have also been demonstrated but the three-way interaction is not yet fully understood and several studies with pigs have failed to show significant reductions in tissue Cu levels from high dietary intakes of Mo and S (Dale et al., 1973, and Kline et al., 1973, cited by Underwood, 1979). Underwood (1979) also adds that Mo can interfere with Cu metabolism at well below 5 ppm Mo or more that have generally been used under experimental conditions or that occur in molybdenosis areas. For example, Suttle (1974) repleted a group of hypocupremic ewes with a

semi-purified diet containing 8 mg Cu/kg and one of four dietary Mo levels, 0.5, 2.5, 4.5 and 8.5 mg/kg. Using rate of recovery in plasma Cu as a measure of the efficiency of Cu utilization, the successive increments in dietary Mo decreased that efficiency by 40, 80 and 40%, respectively. These results indicate that differences of 1 ppm in dietary Mo are of significance with respect to Cu utilization by ruminants. Essentially similar observations were made with guinea pigs. Where dietary Cu intakes are high, more Mo is required to induce hypocupremia. This has drawn attention to the importance of the dietary Cu:Mo ratio. Miltimore and Mason (1971, cited by Underwood, 1979), on the basis of Canadian experiments with cattle, reported that the critical Cu:Mo ratio in animal feeds is 2:1 and that feeds or pastures with lower ratios than 2:1 would be expected to result in a conditioned Cu deficiency. Alloway (1973) reported the results of a study of English pastures which also reveal the importance of the Cu:Mo ratio to the incidence of hypocuprosis in sheep and suggest that the critical ratio is even higher than 2:1, perhaps nearer 4:1.

The first indication of an essential role for Mo in animals came from the discovery that the flavoprotein enzyme, xanthine oxidase, contains Mo and that its activity depends on the presence of this metal. Also, aldehyde oxidase and sulfite oxidase are Mo-containing metalloenzymes (Underwood, 1981). A primary Mo deficiency in commercially fed farm animals or in grazing livestock has never been reported. Such a deficiency seems unlikely because of the low Mo requirements of animals, despite the fact that large areas of Mo-deficient soils exist in which yield responses to applications of Mo occur in crops and pastures (Underwood, 1981). Herbage in the Netherlands contains up to 5 mg Mo per kg; at prevalent contents of Cu in herbage, such values can be taken to be

harmless for cattle. Higher values may be met on alkaline soils where the pasture is rich in clover and on pasture polluted from industry (CMN, 1973).

Assessment of Cu and Mo status

The determination of Cu in the diet or pasture has limited diagnostic value and can, in fact, be seriously misleading unless other elements with which Cu interacts, particularly Mo and S, are determined also. The criteria most widely used for Cu deficiency are the concentrations of Cu in the liver and the blood (Underwood, 1981). Plasma Cu can indicate a deficiency but does not reflect higher "marginal safety" liver storage (Hartmans, 1973, cited by Miller and Stake, 1974). The Cu status of plasma from cattle, sheep and swine can be readily ascertained from serum ceruloplasmin activity. Ceruloplasmin, a true oxidase enzyme (ferroxidase) synthesized in liver, contains up to 8 Cu atoms/mole, depending upon species. In sheep, cattle and swine, a high percentage of the plasma Cu exists as ceruloplasmin, with high correlations between serum Cu and ceruloplasmin activity.

The normal range of blood plasma Cu for sheep and cattle is 0.6 to 1.5 µg/ml (Underwood, 1971). Values consistently below 0.6 µg/ml indicate Cu deficiency in ruminants. Anemia is a common expression of Cu deficiency in all species where the deficiency is severe or prolonged. In these circumstances, the blood Cu in mammals falls as low as .1-.2 µg/ml where normal hematopoiesis cannot be sustained. Copper does not appear to be involved in the heme biosynthetic pathway but is essential for the absorption of Fe from the intestinal mucosa, the mobilization of Fe from the tissues and its utilization in hemoglobin synthesis. These functions are accomplished by ceruloplasmin. This enzyme is necessary

for the formation of Fe (III) transferrin, the transport vehicle of Cu (Underwood, 1981).

Other methods such as histological examination of the tibia (Suttle et al., 1972) or cardiovascular tissues can be sensitive indicators of the Cu status of cattle, sheep and swine. However, in large animals, they are not very useful in detecting early Cu deficiency stages as animals must be biopsied or sacrificed.

According to Cunha et al. (1964), normal Cu concentration of whole blood in the healthy mature bovine is 75 to 100 µg/100 ml. The CMN (1973) indicates that plasma Cu concentrations of 60 to 75 µg/100 ml are considered slightly deficient while levels below 40 µg/100 ml are clearly deficient. For blood serum, the critical Cu values are to be multiplied by .85 to .9. Claypool et al. (1975) found a positive relationship between liver and plasma Cu concentrations. They suggested that liver Cu on the order of 40 ppm was necessary to maintain plasma Cu of 91 µg/100 ml. Plasma Cu levels below 50 µg/100 ml were suggestive of low liver Cu concentrations.

Liver Cu and Mo. The liver is the main storage organ of the body for Cu so that liver Cu concentrations would be expected to provide a useful index of the Cu status of the animal. Liver Cu values vary greatly with the species and age of the animal and in certain disease states, and also with the nature of the diet. Among domestic livestock, liver Cu values are consistently high in healthy sheep, cattle and ducks, with a normal range of 100-400 µg/g on the dry basis, with a high proportion of values lying between 200 and 300 µg/g. In sheep and cattle, liver Cu concentrations vary only slightly from birth to maturity whereas in pigs, they decline with age (Underwood, 1981). Liver Cu concentrations reflect

the dietary status but they are influenced by the dietary proportions of Mo and S, by high intakes of Zn and $CaCO_3$ and other dietary components as well. Animals are able to store large reserves of Cu in the liver. The Cu, upon reaching the liver, which is the principal organ involved in the metabolism of this element, is incorporated in the mitochondria, microsomes, nuclei and soluble fraction of the parenchymal cells in proportions that vary with age, strain and Cu status of the animal. The CMN (1973) reported that the approximate normal level of Cu in cattle liver is 200 ppm on a dry matter basis. Levels below 50 ppm indicate a deficiency and below 10 ppm, extreme deficiency. The Cu levels in the livers of young bulls on native pasture, with and without complete mineral supplementation, were 342 and 232 ppm on a dry matter basis, respectively, as reported by Lebdosoekojo (1977). Underwood (1979) reported that a forage Cu level of 8 to 10 ppm (dry matter basis) can produce chronic Cu toxicity in sheep and cattle when the concurrent Mo plus S dietary levels are abnormally low (.2 ppm Mo or less). Generally, liver Cu concentrations in the majority of animal species decrease as the animal matures; however, in cattle there is little variation between young and mature animals, although sometimes young animals may have higher Cu concentrations than adults (Church, 1971).

In sheep and cattle, liver Cu concentrations are influenced by various dietary factors and can be reduced by increasing Mo and S dietary levels. Standish et al. (1971) reported that high dietary Fe depressed absorption of Cu. Sheep fed a basal diet containing 77 ppm Cu supplemented with 0, 400 or 1600 ppm Fe in the form of ferrous sulfate showed Fe concentrations in the liver of 185, 269 and 605 ppm, respectively, while the corresponding liver Cu levels were 260, 145 and 44 ppm. Cattle

grazing on acid soils had liver Cu contents of 6 ppm (dry matter basis), yet none showed signs of ill health, as reported by Bingley and Anderson (1972). The same authors reported levels of 2 ppm Cu in pastures grazed by calves with falling disease in Australia and found liver Cu levels of 1.6 to 6.7 ppm (dry matter basis) in dead animals. Rosa (1980) studied the interrelationship between Cu, Zn and Fe using Florida native mature wethers and found that there was a main effect of high dietary Cu on liver Cu concentration represented by an increase ($P < .01$) of hepatic Cu from 341 to 850 ppm. Liver Cu was also affected by an interaction ($P < .05$) of Fe by Zn. Both high dietary Zn and Fe increased liver Cu concentration in the presence of low levels of the other element.

The tolerance of farm animals to high dietary Mo intake varies with the species, the amount and chemical form of the ingested Mo, the Cu status of the animal and the diet, and the S content of the diet and its content of substances such as protein, methionine and cystine capable of oxidation to sulfate in the body. Cattle are by far the least tolerant species, followed by sheep, while horses and pigs are the most tolerant of domestic livestock. In normal diets, the level of Mo in the liver is of the same order, namely 2-4 ppm, in several species of widely differing dietary habits. Similar concentrations occur in the livers of newborn lambs, indicating that this element is not normally stored in the fetal liver during pregnancy. However, Mo concentrations 3 to 10 times the normal level were observed in the livers of newborn lambs from ewes receiving a high Mo diet (Underwood, 1971). This author also suggests that Mo readily passes the placental barrier in this species. Adult sheep and cattle retain Mo concentrations in their livers of 25 to 30 ppm as long as they are ingesting large or moderately large amounts of Mo. The

levels rapidly return to normal when the administration of the extra Mo ceases.

Underwood (1971) reported that about one-half to three-fourths of the total body Mo of sheep is situated in the skeleton, with the next largest proportions in the skin, wool and muscles and only about 1% of the total in the liver. This contrasts markedly with the distribution of total body Cu in which a high proportion occurs in the liver in sheep and very little in the skeleton. The author concluded that the Mo level in the liver of an animal, therefore, gives little indication of its dietary Mo status and is of limited diagnostic value for this reason, unless the sulfate and protein status of the diet of the animal is also known.

Hidiroglou et al. (1982) reported bone abnormalities in sheep and cattle from Mo toxicity. Bones of the Mo-supplemented animals contained more Mo than those from nonsupplemented animals. The author also found that the Ca and P contents of sheep bones were unaffected by Mo supplementation.

Cattle are much more subject to Mo toxicosis than are sheep. Signs of molybdenosis include diarrhea and rapid loss of weight. The disease usually occurs on pasture containing 5 to 20 ppm Mo on a dry matter basis but when dietary Cu intake is abnormally low or dietary sulfate intake is high, Mo intake as low as 1 to 2 ppm may be toxic (Pope, 1975). Ward (1978) has reviewed studies that indicated that in the liver, Mo inhibited the oxidation of sulfides to sulfate, resulting in the accumulation of sulfides in the liver and their precipitation as Cu sulfides.

McDowell et al. (1982), studying cattle in Florida, found that mean liver Mo contents were 2.8 and 3.0 ppm during the wet and dry seasons,

respectively. These values are in agreement with approximate normal levels of 2 to 4 ppm indicated by Underwood (1977) and suggest that Mo was not present in high enough concentrations to interfere greatly with Cu metabolism. In a tropical region of South America (Bolivia), Peducassé (1982), working with grazing Zebu-Criollo cattle, found that mean liver Mo content was 4.3 ppm, with a range of 3 to 6.3 ppm. Lebdosoekojo (1977) reported liver Mo levels in young bulls on native pastures with and without mineral supplementation as 3.9 and 4.5 ppm, respectively.

Sousa (1978) evaluated the mineral status of beef cattle in northern Mato Grosso, Brazil, and found that mean liver Mo contents were 2.5 ± .8 and 2.7 ± 1.0 during the dry and wet seasons, respectively. There was a trend to have more than the normal range of liver Mo during the wet season. This increase in liver Mo was due to a moderate increase in forage Mo during the wet season (2.3 ppm vs 1.6 ppm). Levels of Mo up to 40 mg/d tend to increase hepatic Cu levels while dietary Mo levels beyond 40 mg/d may alter the hepatic levels very little (Ammerman and Miller, 1975).

Toxicosis is the major concern in Mo nutrition. Suttle (1980) suggested that values even less than 10 ppm dietary Mo in ruminants affect Cu metabolism. Clinical signs of Mo toxicosis are similar to those of Cu deficiency. Molybdenum-toxic areas characteristically occur on poorly drained neutral or alkaline soils. According to Underwood (1977), Mo toxicity occurs in cattle grazing pastures with 20 to 100 ppm Mo but not in cattle grazing normal pasture with 3 to 5 ppm Mo or less.

Chronic Cu poisoning is the result of continual ingestion of Cu in concentrations that exceed the maximum safe level of 80 ppm. During the time of accumulation of Cu in the liver, clinical signs are absent but later a hemolytic crisis may occur. This crisis is characterized by the

sudden onset of severe hemolysis and hemoglobinemia associated with severe jaundice, liver and kidney damage and rapid death (Brakley et al., 1982).

Zinc and Manganese

Metabolism in ruminants

Zinc had been established as essential for laboratory animals in the 1930's but even in 1956, relatively little was known about its nutrition and metabolism in cattle (W. Miller, 1981). Since 1960, Zn nutrition and metabolism in cattle has been the subject of considerable research. Before its cause was shown to be inadequate Zn, parakeratosis was a major practical problem in swine production. According to the CMN (1973), Zn deficiency has been established as a practical problem in mature cattle only in tropical South America. Signs were poor growth, parchment-like thickening of the skin called elephant skin or parakeratosis especially on the muzzle and limbs but also around the base of the tail, on the neck and flanks, and the skin extremely susceptible to wounds and infections. In the Netherlands, such signs have been observed sporadically in calves.

Zinc is widely distributed throughout the body and plays an essential role in many body processes. It is present in many enzyme systems involved with the metabolism of feed constituents (Cunha, 1981). Zinc metalloenzymes include carbonic anhydrase, alcohol dehydrogenase, alkaline phosphatase, carboxypeptidase, RNA and DNA polymerases, thymidine kinase and others whose structure and functions have been critically reviewed by Riordan and Valle (1976, cited by Underwood, 1981). Zinc was found to play a vital role in DNA synthesis and nucleic acid and protein metabolism so that all systems of the body suffer in Zn deficiency,

particularly when the cells of particular systems are rapidly dividing, growing or synthesizing. For these reasons, growth and reproduction especially are affected by lack of Zn (Underwood, 1981).

The estimated Zn requirement for dairy cattle is 40 ppm in the diet. There may be certain conditions or an interrelationship with other nutrients which might increase Zn needs. For example, a small percentage of Dutch-Friesian calves are born with an apparently inherited defect that causes a very severe Zn deficiency which can be temporarily corrected by high amounts of Zn (Cunha, 1981). Animals are quite tolerant of excessive dietary Zn. The first effects are lower feed consumption and reduced weight gains. Excessive Zn interferes with the metabolism of some other trace elements, especially Cu and Fe. Except where massive amounts of Zn are fed either intentionally or accidentally, it appears unlikely that too much Zn should be a practical problem (W. Miller, 1981).

Studies on excess Zn levels indicate that lactating dairy cows fed 1279 ppm of Zn in the diet did not experience reduced performance. Growing cattle fed 900 ppm Zn exhibited decreased weight gains and feed efficiency (Cunha, 1981). On the basis of these studies, the 1978 NRC publication on nutrient requirements of dairy cattle gives an estimated safe level of 500 ppm Zn in young cattle and 1000 ppm in older cattle. The Zn requirement for sheep is 35-50 ppm in the diet; the toxic level is 1000 ppm (NRC, 1975). The major homeostatic control route of Zn is a variable percentage of absorption. Variable endogenous fecal excretion, but not urinary excretion, also contributes to Zn homeostasis (W. Miller, 1981). Zinc levels of 1.7 g/kg of diet and higher caused reduced feed consumption and depraved appetite characterized by excessive salt and other mineral consumption and wood chewing in beef cattle (Ott et al.,

1966b) while Zn consumption above 1.5 g/kg of diet caused depressed feed consumption in lambs (Ott et al., 1966a). The author also reported that water consumption was also suppressed by force feeding 4.0 to 6.0 g Zn daily. No other external symptoms of the toxicity were observed but prolonged consumption of high levels of Zn caused death.

Manganese was first recognized as an essential mineral element for animals in 1931 when it was shown to be required by rats and mice for growth and reproduction (Perry, 1981). The enzymes that are activated by Mn are numerous and include kinases, hydrolases, transferases and decarboxylases. Activation was found usually to be shared with other bivalent cations, notably Mg (Valle and Coleman, 1964, cited by Underwood, 1981). A specific function for Mn in the synthesis of the mucopolysaccharide has been demonstrated. It appears that Mn functions in the metabolism of carbohydrates and lipids are very important. The mitochondria normally contain high levels of the element as do pyruvate carboxylase and superoxide dismutase (Perry, 1981).

Manganese is poorly absorbed and excreted mainly in the feces, with absorbed Mn also appearing in the feces, mostly via the bile and pancreatic juices. High dietary intakes of Ca and Fe reduce Mn absorption and different mineral sources of the element vary greatly in their availability. The animal body has only a limited capacity to store mobilizable reserves of Mn. The bones, liver and kidneys normally carry higher concentrations of Mn than the blood or muscles and the former can be raised or lowered by substantially increasing or decreasing the Mn intake of the animal (Hidiroglou, 1979).

Although progress has been substantial, current information on nutrition and metabolism of Mn for dairy cattle is still incomplete (W. Miller,

1981). The author also reported that relatively large amounts of Mn are present in most soils and in most feed ingredients. The percentage of dietary Mn absorbed is low (typically around 3 to 4%) and variable, depending on the dietary content. Manganese deficiency had not been produced in sheep or goats until 1968 when early weaned lambs receiving a purified diet containing less than 1 ppm Mn over a 5-month period exhibited bone changes similar to those seen in other Mn-deficient animals. The exact requirements of sheep for Mn are not known (NRC, 1975).

General symptoms of Mn deficiency include impaired growth, skeletal abnormalities, disturbed or depressed reproduction and abnormalities (including ataxia) of the newborn (Underwood, 1971). In cattle, the Mn requirement is substantially higher for reproduction and birth of normal calves than for growth (NRC, 1978). Rojas et al. (1965) found in one experiment that all calves born from cows fed 16-17 ppm dietary Mn for a 12-month period had neonatal deformities. Heifers and cows fed low Mn diets are slower to exhibit estrus, are more likely to have "silent heats" and have lower conception rates (NRC, 1978).

Assessment of Zn and Mn status

In feeding trials with Zn-deficient diets, signs appear rapidly, sometimes within a week, in young cattle. Before they appear, concentration of Zn in blood plasma falls. Young cattle seem to be directly dependent on supplies in the ration and not to hold any significant mobilizable reserve. Zinc concentration in plasma of healthy cows is .60 to 1.40 mg/liter. Immediately after calving, values may fall to about .50 mg/liter. For clinical signs, values are usually less than .40. Repeatedly low concentrations in plasma are a reasonable criterion for determining Zn status of the animal but values can fluctuate rapidly and are greatly affected by infection or poor food intake (CMN, 1973).

Zinc is present in the blood plasma, erythrocytes, leucocytes and platelets. Almost all of the Zn in erythrocytes occurs as carbonic anhydrase. Subnormal carbonic anhydrase activity occurs in the blood of Zn-deficient calves. A decline in plasma or serum Zn has been observed in deficient animals of all species studied (Underwood, 1981). Mills et al. (1967) reported a fall from normal values of .8-1.2 µg Zn/ml to below .4 µg/ml in the blood serum of severely deficient lambs and calves.

Under experimental conditions, many biochemical changes have been identified in severely Zn-deficient animals. Those with most promising diagnositc value are plasma (or serum) Zn, hair Zn, bone Zn and alkaline phosphatase content of plasma or other tissues (Miller and Stake, 1974). These authors suggested that alkaline phosphatase is substantially affected by numerous other factors, with large individual variability within herds and large mean difference in similar herds. The Zn content in male sex organs and secretions, which are also normally high, similarly reflect the status of the animal. Values of 105 ± 44 and 74 ± 5 ppm Zn (dry matter basis) were reported for the testes of normal and Zn-deficient rams, respectively (Underwood and Somers, 1969, cited by Underwood, 1981). Plasma Zn has been reported to decrease during parturition in the cow (Prior, 1976; Dufty et al., 1977) and to decrease more in cows with dystocia than in normal cows (Dufty et al., 1977). In contrast to plasma Zn, which tends to reflect dietary changes, hair Zn is reduced when a Zn-deficient diet is fed over a period of time (Miller et al., 1965b). However, hair Zn is affected by many other factors; thus, under practical conditions, its diagnostic value is severely limited.

According to the CMN (1973), there is as yet no practical criterion for assessing the Mn status of animals. Liver biopsy seems the most

promising. Analysis of hair is useless since results are difficult to interpret. Decreased bone and blood alkaline phosphatase have been observed in Mn deficiency (Underwood, 1966). However, as discussed with Zn, alkaline phosphatase is affected by many conditions and thus is not a good biochemical measurement of Mn deficiency (Miller and Stake, 1974). Blood Mn values are extremely variable, reflecting both individual variability and analytical inadequacies. Whole blood concentration substantially below .02 µg Mn/ml, nevertheless, suggest the possibility of a dietary deficiency in sheep and cattle, according to Hidiroglou (1979). The Mn contents in wool and feathers apparently reflect the dietary status of the animals but their diagnostic value is doubtful, at least at marginal intakes. However, the wool of lambs fed a low Mn diet for 22 weeks had an average of only 6.1 ppm Mn compared with 18.7 ppm in the wool of control lambs (Lassiter and Morton, 1968, cited by Underwood, 1981). Manganese is distributed throughout the body and is found in higher concentrations in bone, liver, kidney and pancreas (McDowell et al., 1978).

The Mn concentration in the whole diets remains the most useful means of detecting possible deficiency in animals. According to the NRC (1976), most of the roughages contain more than 30 ppm Mn. Thomas (1970) found interrelationship of Mn with other dietary factors such as organic compounds, Ca, P, Mg and Fe. Protein concentrates of animal origin such as meat meal and fish meal are poorer sources of Mn (5--15 ppm) than the usual protein supplements of plant origin such as soybean meal (30--50 ppm). Milk and milk products are even lower in this metal due to the generally very low content of Mn in cow's milk (20--40 mg/liter), according to Underwood (1981).

Liver Zn and Mn. Zinc content of tissues other than plasma decreases quite slowly, if at all, when a Zn-deficient diet is fed (Miller, 1969). Zinc content in a number of calf tissues, including liver, kidney and pancreas, was increased several fold when 600 ppm supplemental Zn was fed and before any symptoms of toxicity appeared (Miller et al., 1970). Factors which reduce Zn absorption in the gastrointestinal tract have also been indicated to reduce Zn concentration in the liver. Standish et al. (1971) reported that Fe fed to cattle at high levels of 400 to 1600 ppm tended to decrease liver Zn concentrations; moreover, Cd, Ca, Mg, P and Cu, as well as chelating agents such as EDTA, vitamin D and phytic acid, influence Zn absorption and metabolism (Miller, 1972). Underwood (1962) suggested that liver Zn values above 125 ppm should be considered as the normal value for cattle. Neathery et al. (1973) reported tissue Zn concentration as affected by dietary Zn of 16.6 and 39.5 ppm in the dry diet and found liver Zn levels of 109 and 119 ppm, respectively. Watson et al. (1973) also found lowered Zn concentrations in the liver of sheep fed high dietary Mn levels. The binding pattern of liver Zn, like the total Zn concentration, was unaltered in Zn deficiency; therefore, for detecting mineral status of animals, Zn in liver is not a good indicator. Other tissues such as bones, pancreas, the male sex glands, hair and blood plasma are better indicators of the mineral status in cases of Zn deficiency.

As far as Mn is concerned, liver can be used as a criterion to differentiate between deficient and sufficient supply of the element. The approximate normal level of Mn in cattle liver is 8 to 10 ppm (dry matter basis); below 8 ppm Mn indicates deficiency (Underwood, 1977). McDowell et al. (1978) suggests that a Mn deficiency can best be detected

by the combination of liver (less than 6 ppm Mn) and dietary (less than 20 to 40 ppm) analyses. The bones, liver, kidney, pancrease and pituitary gland normally carry higher Mn concentrations (1-3 ppm on fresh basis) than do other organs. The skeletal muscles are among the lowest in Mn (.1-.2 ppm) of the tissues of the body. The levels in the bones can be raised or lowered by substantially varying the Mn intake of the animal. The storage capacity of the liver for Mn is limited, compared with the great capacity of this organ to accumulate Fe and Cu (Underwood, 1971).

Kiatoko (1979) found that hair Mn was negatively correlated with liver Mn, indicating that hair is not a good indicator of Mn status. Rojas et al. (1965) fed low Mn (15.8 to 16.9 ppm) diets to 6 to 8-year old cows. All calves born to deficient dams exhibited clinical signs of deficiency of Mn. Liver Mn concentrations in control and deficient calves were 11.84 and 6.94 ppm on a dry matter basis, respectively. Under such conditions, 25 ppm Mn in the diet was considered marginal and liver Mn levels of 9 ppm by dry weight indicated a borderline deficiency. Finally, Watson et al. (1973) reported an increased liver Mn concentration in sheep from 9.9 to 44.2 ppm as the dietary Mn increased from 30 to 4030 ppm.

Cobalt

Metabolism in ruminants

Cobalt was first shown to be an essential nutrient for sheep and cattle as an outcome of Australian investigations of two naturally occuring diseases, "coast disease" of sheep and "wasting disease" or enzootic marasmus of cattle (Underwood, 1981). Progress in understanding the mode of action of Co in the animal organism was slow until 1948 when

two groups of workers independently discovered that the antipernicious anemia factor, subsequently designated as vitamin B_{12}, is a Co compound containing 4% of the metal. Cobalt is an essential component of vitamin B_{12} which is synthesized by rumen microorganisms. Cobalt should be fed since injected Co is completely effective in alleviating Co deficiency symptoms.

The minimum Co requirement of dairy cattle is about 0.10 ppm of the dry ration (Ammerman, 1970; Underwood, 1971). Since the required level is more than the amount contained in many forages and some concentrates, supplemental Co is needed under many practical situations. The main source of energy to ruminants is not glucose but acetic and propionic acids and smaller amounts of butyric and other fatty acids produced by fermentation in the rumen. Any breakdown in the utilization of these acids involving vitamin B_{12} would therefore seriously affect the Co-deficient ruminant. A breakdown in propionate metabolism at the point in the metabolic pathway where methylmalonyl-CoA is converted to succinyl-CoA, a reaction catalyzed by methylmalonyl-CoA isomerase, a vitamin B_{12}-requiring enzyme, has been shown to be a primary defect in the Co-deficient sheep (Marston et al., 1961, cited by Underwood, 1981). A severe Co deficiency causes appetite failure which is due, at least in part, to the animal's inability to metabolize propionate. This is followed by the onset of anemia and eventually, extreme emaciation. Vitamin B_{12} has also been shown to exert a potent influence on the recycling of methionine and via methionine, on folate metabolism. This occurs through the activity of a second vitamin B_{12}-containing enzyme, 5-methyltetrahydrofolate homocysteine methyltransferase, which catalyzes the reformation of methionine from homocysteine. Therefore, the activity of this

methyltransferase is depressed in the liver of vitamin B_{12}-deficient sheep, with possible impaired nitrogen retention (Gawthorne and Smith, 1974, cited by Underwood, 1981).

Assessment of Co status

Young, growing sheep are the most sensitive of all animals to Co deficiency; next are mature sheep, calves between 6 and 18 months of age and mature cattle. Cobalt deficiency occurs in many regions of Latin America and mostly, but not exclusively, is restricted to grazing ruminants which have little or no access to concentrates (McDowell and Conrad, 1977). According to the CMN (1973), the Co concentration in tissues is too low for easy estimation as a criterion of Co status. Because of this and the lack of specific clinical signs, little is known about the effect of nutrition and environmental conditions on Co status. The best method of tracing deficiency is to give cattle a supplement of Co salts and to see how they react.

Effective biochemical diagnostic measures for Co deficiency have been developed (Underwood, 1966, 1971). Since this is an area problem, knowledge of the soil (Co, pH, etc.) is very useful. The Dutch consider soil Co of greatest diagnostic value. When Co is extracted with acetic acid (2.5% v/v), more than .3 ppm indicates adequate Co whereas less than .1 ppm is low. Under most conditions, mean pasture values of .10 ppm Co or more supply sufficient Co to prevent deficiency. However, if the herbage consistently contains less than .08 ppm Co, a deficiency can be predicted with a relatively high degree of confidence (Conrad, 1978).

In Queensland, Australia, Winter et al. (1977) reported that the occurrence of Co deficiency in grazing animals was related to the presence of sandy soils low in Co content and to the seasonal variation of

Co concentration in forages; during the wet season, they found forages with .126 ppm Co while in the dry season, levels decreased to .005 ppm. Sousa (1978) reported that most of the native forages in the lowlands of Mato Grosso, Brazil, had mean levels in the range of .04 to .14 ppm Co. Research reviewed by Ammerman (1970) suggested that hair Co levels might also have some value in predicting the animal's Co status. Serum methylmalonate and fleece growth rates are more reliable indicators of Co deficiency in sheep than urinary methylmalonate (Judson et al., 1981). The authors also reported that liveweight and wool growth responses to Co pelleted therapy in weaner sheep of South Australia were useful.

Liver Co and vitamin B_{12}. Cattle and sheep with normal stores of vitamin B_{12} in their livers can consume a Co-deficient diet for months without showing any signs of a vitamin B_{12} deficiency. Reduced ruminant liver stores of Co and vitamin B_{12} are indicative of a dietary Co deficiency and storage levels are frequently used to determine the Co status of ruminants (Ammerman, 1981). Based on a study in New Zealand, Underwood (1971) has indicated that Co concentrations of .04 to .06 ppm or less in the liver of cattle and sheep indicate Co deficiency. Cunha et al. (1964) suggested a liver Co level of .04 ppm indicated extreme deficiency. Most studies indicate that cattle and sheep liver Co levels below .1 ppm on a dry basis are low to deficient while levels between .15 and .30 ppm are normal for healthy animals (Conrad, 1978). Liver vitamin B_{12} concentration is a more sensitive and reliable criterion than liver Co concentration.

Andrews (1960, cited by Miller and Stake, 1974) adopted the following criteria for determining the Co status of sheep: values of vitamin B_{12} in fresh liver below .07 ppm are considered to indicate severe Co

deficiency; between .07 and .10 ppm B_{12}, moderate Co deficiency; between .11 and .19 ppm B_{12}, mild deficiency; and values over .19 ppm B_{12} considered adequate.

Cobalt is known to interact with Fe, Cu, Se and Mo. An interrelationship has been suggested between Co and Se. Cobalt-deficient sheep are more susceptible to Se toxicity than sheep fed adequate Co (Ammerman, 1981). It is suggested that the Co-Se relationship may involve vitamin B_{12} in the metabolism of dimethyl selenide. The NRC (1976) has recommended a level of .05 to .10 ppm Co in the diet. Mendes (1977) reported mean liver Co values ranging from .040 to .691 ppm in the wet season and .124 to .730 ppm in the dry season in 5 classes of cattle in northern Mato Grosso, Brazil. Kiatoko (1979), in cattle of Florida, found that liver Co concentration was 1.06 ppm in cows and .88 ppm in heifers where forage Co levels varied from .09 to .12 ppm in the wet season and from .12 to .26 ppm during the dry season. Peducassé (1982), studying cattle in Bolivia, found that mean liver Co concentrations were .57 and .30 ppm in the two regions sampled; none of the liver samples contained levels below the .05 ppm cited by McDowell and Conrad (1977) as borderline to deficient for grazing cattle. Jerez (1982) studied the mineral status of grazing cattle in three regions of the Dominican Republic and found that liver Co concentrations for regions 1, 2 and 3 were .39, .42 and .65 ppm, respectively. Mtimuni (1982), working in Malawi, reported that Co was not deficient in the liver samples analyzed.

Selenium

Metabolism in ruminants

Selenium was first recognized as an essential trace mineral in 1957 when it was found to prevent liver necrosis in rats. Subsequently, Se

deficiency affecting a number of systems and producing a variety of lesions has been observed in swine, poultry, horses, sheep and cattle (Hidiroglou, 1980). In cattle, white muscle disease is the most commonly recognized problem. For many years, biological interest in Se was confined to its toxic effects on animals. Two naturally occurring diseases of livestock, "blind staggers" and "alkali disease," observed in parts of the Great Plains of North America, were identified as manifestations of acute and chronic Se poisoning, respectively. These discoveries gave a stimulus to investigation of Se in soils, plants and animal tissues with a view to determining minimum toxic intake and developing practical means of prevention and control (Underwood, 1981).

Selenium is necessary for growth and fertility in animals and for the prevention of a variety of disease conditions which show a variable response to vitamin E. A metabolic interrelationship between Se, vitamin E and S-containing amino acids exists at the cellular level. Selenium functions in the cytosol through glutathione peroxidase (GSH-Px). Glutathione peroxidase uses glutathione, a tripeptide with a S, to reduce hydrogen peroxide and organic hydroperoxides to less harmful products (Hidiroglou, 1980). Also, glutathione peroxidase, through its role in the metabolism of hydroperoxides, may be involved in the synthesis of various prostaglandin derivatives. Vitamin E acts as a lipid soluble antioxidant in the cell membrane.

The metabolism of absorbed Se appears to be similar for ruminants and nonruminants. A portion of dietary Se becomes incorporated into microbial material. The major excretory pathway for oral Se is fecal in ruminants and urinary in nonruminants under most conditions (Martin and Gerlach, 1972). In the younger preruminant lamb or calf, Se deficiency

exerts damaging effects more frequently than in the older animal. With development of the rumen, affected animals may recover from NMD (Whanger, 1970, cited by Ammerman et al., 1978). The effects of vitamin E and Se deficiency have been postulated to result from loss of membrane integrity which leads to cell death. Addition of polyunsaturated fatty acids to the diet tends to exacerbate these deficiency defects whereas synthetic antioxidants, in many cases, will alleviate the signs of vitamin E and Se deficiency. Selenium was also classified as an antioxidant due to its ability to prevent a number of vitamin E deficiency diseases (Sunde and Hoekstra, 1980). The authors also suggested that in a typical animal cell, lipid-soluble α-tocopherol scavenges free radicals and possibly quenches singlet oxygen in the membranes. GSH-Px and superoxide dismutase react with peroxides and superoxide, respectively, in the cytosol and mitochondrial matrix space and catalase destroys H_2O_2 in the peroxisomes. The relative concentration and the importance of these protective species vary from tissue to tissue and from specie to specie and result in the variety of Se and/or vitamin E deficiency signs observed in different species.

Vitamin E and Se seem to have an additive effect on the reduction of serum glutamic oxalacetic transaminase (SGOT) activity, increasing survival time of the lambs and decreasing the ratio of urinary creatine to creatine excretion in lambs less than 8 weeks old. Thus, the need for vitamin E in the diets of nursing lambs is related to Se in the diet and vice versa (Pope, 1975).

Failure in reproductive function and a high incidence of retained placentas have been associated with Se-deficient rations. In some studies, supplementation with Se, Se-vitamin E or Se and increased protein,

significantly reduced the incidence of retained placenta (Hidiroglou, 1980). The dietary requirement of Se by most species is considered to be about .1 ppm. In grazing animals, three distinct Se deficiency syndromes have been described: "white muscle disease" (WMD) in newborn or young lambs and calves; unthriftiness, with poor growth rates which may occur in the absence of any other recognizable disease; and infertility (McDowell, 1978).

Assessment of Se status

The most widely used assessment of Se status is blood Se concentration. Low blood Se is always found in Se-deficient conditions. A direct relationship between blood GSH-Px activity and Se concentrations has been established (Hidiroglou, 1980). The author also reported that Se is incorporated into the erythrocyte GSH-Px at the time of erythropoiesis; thus GSH-Px levels are less affected by daily variations in the dietary level. In two experiments reported by Kuchel and Buckley (1969, cited by Underwood, 1971), the concentration of Se in the whole blood of sheep grazing pastures of normal Se status ranged from .06-.20 (mean .10) µg/ml in one study and from .04-.08 (mean .06) µg/ml in the second experiment. The administration of Se pellets induced a rapid rise in blood Se to levels as high as .15-.25 µg/ml, depending on the amount of Se in the pellets. Segerson and Johnson (1980) studied the effect of Se and reproductive function in yearling Angus bulls and found pooled Se at 21-day intervals averaged .01 and .08 ($P < .001$) for control and Se-supplemented bulls, respectively. They concluded that injections of supplemental Se increased both serum and tissue concentrations of this element. No overt clinical signs of Se deficiency were observed in control bulls even though serum Se concentrations were as low as .01 ppm. In contrast,

the serum Se concentration (.08 ppm) for treated bulls was comparable to serum levels considered adequate for various aspects of reproductive performance in beef and dairy cows. In this study, Se in serum was correlated ($P < .05$) with Se in kidney, liver, seminal vesicle and testis ($P < .10$) tissues.

Since Se is deposited in all the tissues of the body, except the fat, of animals consuming seleniferous feeds, high concentrations of the element provide indisputable evidence of an excessive intake. The Se content in urine, blood and hair similarly reflect dietary intakes but are highly variable. Hair from normal cows generally contains 1-4 ppm Se compared with 10-30 ppm for cattle on seleniferous range (Underwood, 1981). The concentration of Se in milk varies greatly with the Se intake of the animal. Perry et al. (1977) observed milk Se concentrations ranging from 7 to 33 ppb when cows were supplemented with linseed meal. Conrad and Moxon (1979) found that 4.8% of supplemental Se was transferred to milk when animals were fed a Se-deficient diet but that only .9% of added Se was transferred to the milk of cows consuming diets adequate in Se. Ammerman et al. (1980) found that milk Se concentrations, which ranged from 7 to 20 ppb, were higher ($P < .01$) for cows on the linseed meal plus Se treatment than for those receiving no Se supplementation. Sousa and Moxon (1982) studied the serum Se levels in cattle from Brazil and found serum Se deficiency ($\leq .02$ ppm) in grazing dairy cows. One calf was observed with white muscle disease (WMD) with low serum Se levels (.005 ppm). Mans et al. (1980) reported that plasma Se adjusted slowly and latently when increased Se was fed and that Se accommodation lasted for 7 weeks or more.

Liver and kidney Se. The kidney and the liver are the most sensitive indicators of the Se status of the animal and the Se concentrations in these organs can provide valuable diagnostic criteria. Andrews et al. (1968, cited by Underwood, 1971) suggested that Se levels of less than 0.25 ppm are indicative of marked Se deficiency in sheep. Concentrations greater than 1.0 ppm Se in the kidney cortex and .1 ppm in the liver are considered normal and one-half the quantity of these levels indicates a marginal degree of Se-responsive unthriftiness. McDowell et al. (1978) indicated that normal Se levels in the liver of cattle are usually above .25 to .50 ppm (dry matter basis) while values on the order of 5 to 15 ppm (dry matter basis) are suggestive of an excessive Se intake. Selenium (ppm) in kidney and liver tissues of control and Se-treated bulls, respectively, was .84 and 1.27 ($P < .005$) and .1 and .37 ($P < .001$) (Segerson and Johnson, 1980). Ammerman et al. (1980) found that liver concentrations were higher ($P < .05$) for calves from cows fed linseed meal calculated to provide a natural Se adequate level than they were for calves from cows fed the natural, Se-deficient soybean meal supplement.

Kiatoko (1979), in Florida, reported liver Se concentrations were below critical levels (< .25 ppm) in 32.2 and 38.8% of the samples in the wet and dry seasons, respectively. Like forage and soil Se, liver and hair Se concentrations were deficient. Peducassé (1982) reported a mean of .70 ppm in cattle from tropical areas of Bolivia. McDowell et al. (1982) studied trace mineral status of cattle in Florida and concluded that the most pronounced finding from the analysis of samples in all regions was the low Se status of pastures, soils and animal tissues. No effect of Se administered in mineral supplements existed as samples were

collected before the Food and Drug Administration approved dietary additions of this element. They suggested the need for increasing dietary intake of Se because of persistent reports of white muscle disease in Florida.

Much of the Se in tissues is highly labile and transfer of animals from seleniferous to nonseleniferous diets is followed by rapid and then slow loss of Se from the tissues via bile, urine and/or expired air. Concentration in tissues tends to reflect dietary Se concentrations, particularly when provided by natural dietary ingredients as compared to selenate or selenite (NRC, 1980). Ku et al. (1980, cited by NRC, 1980) found the Se concentration of swine skeletal muscle (.034-.521 ppm, wet basis) was highly correlated ($r = .95$) with that in natural swine diets (.027-.493 ppm, air dry) from 13 different U.S. locations. When these workers added sufficient Se (.4 ppm) from sodium selenite to raise a low Se (.04 ppm) swine diet to the level found in a South Dakota swine diet (.44 ppm from natural sources), respective skeletal muscle Se concentrations were .12 and .48 ppm, wet basis. Corresponding liver Se concentrations were .61 and .84 ppm, wet basis. A similar pattern of tissue Se concentrations has been found in cattle and sheep (Ullrey et al., 1977). McDowell et al. (1977) reported that supplementation of a basal ration with .10 ppm Se as sodium selenite resulted in an eightfold increase in hepatic Se, nearly a fivefold increase in renal cortical Se and a 25-fold increase in blood Se concentrations compared to pigs fed the unsupplemented basal ration. Also, supplementation of the basal ration with 100 ppm vitamin E resulted in increased renal cortical ($P < .01$) and blood ($P < .05$) Se concentrations. The authors finally suggested that hepatic, renal cortical and blood Se concentrations of .25, 2.5 and .1 ppm (dry

basis), respectively, were determined to be the critical levels below which clinical illness, death or lesions of Se-vitamin E deficiency could be expected.

Although Se concentration in plasma and liver provide the best indicator of current dietary Se intake in cattle, erythrocyte GSH-Px activity is suitable as an alternative test for the routine diagnosis of Se deficiency. Also, there is a clear relationship ($r = .97$) between blood Se concentration and erythrocyte GSH-Px activity in samples tested from 50 mixed age Friesian/Jersey cattle in New Zealand (Thompson et al., 1981).

Energy and Protein

Insufficient energy probably limits performance of sheep more than other nutritional deficiencies and may result from inadequate amounts of feed or from feed of low quality (NRC, 1975). Energy, the principal dietary constituent, generally represents between 70 and 90% of the daily dry matter intake. In young animals, an insufficient supply of energy results in retarded growth and delay in the onset of puberty; in lactating dairy cattle, it results in a decline in milk yield and loss of body weight. Severe and prolonged energy deficiency depresses reproductive function. There is a close relationship between energy and minerals in the metabolism of the animal body. For example, during glycolysis, the anaerobic degradation of glucose to yield lactic acid, there are different metabolic pathways, in which different enzyme systems prevail. Phosphorus, part of the high-energy ATP, is the first utilized during the two priming steps of glycolysis. The phosphorylation of D-glucose at the 6 position by ATP to yield D-glucose 6-phosphate is catalyzed by two types of enzymes, hexokinase and glucokinase, which differ in their sugar

specificity and affinity of D-glucose. Both hexokinase and glucokinase require a divalent cation (Mg^{2+} or Mn^{2+}) (Lehninger, 1975).

Energy requirements for breeding cattle are 1.9 Mcal metabolizable energy (ME) or 2.3 Mcal digestible energy (DE) per kg dry matter (NRC, 1976). For grazing livestock in tropical areas, forages are the major source of the essential nutrients of energy, protein, vitamins and minerals. Butterworth (1964) reported the values of digestible energy of 21 tropical grasses to range between 2.23 and 3.20 Mcal per kg. The same author (Butterworth, 1967) also reported that the availability of energy and protein as measured by their apparent digestion by sheep and cattle declines rapidly with advancing maturity. Minson (1980) worked with tropical forages in Australia and reported that voluntary intake of digestible organic matter (DOM) is an acceptable expression of forage quality because it is closely related to digestible energy (DE) intake and to animal performance. Voluntary intake and nutrient digestibility must be considered separately because they are often not closely related across species. In a study of 41 southern forages (Moore et al., 1980), the correlation (r) between dry matter intake and digestibility was .69. As they advance beyond a few weeks growth, most tropical forages have high lignin content which influences digestibility and feed intake. Moore and Mott (1973) reported that the crude protein percentages should be examined first when an explanation of an unexplained low production is observed before looking for other limitations such as those related to forage structure, other nutrients and toxic effects. Also, the authors suggest that lignin must be considered as the primary structural inhibitor of quality in tropical grasses within a given species. Using percentage of crude protein (CP) and in vivo digestible organic matter, Golding (1976) developed the

following equation to predict digestible energy (DE) concentration of warm seasonal grasses:

$$DE = (4.15\ DOM + 1.200\ CP - 4.59) \times 1/100$$

Supplementation of low protein forages (less than 7% crude protein, dry matter basis) with protein may increase voluntary intake (Ventura et al., 1975). With respect to supplemental energy, the interaction is even more complex. There are two extreme effects, substitutive or additive (Moore and Mott, 1973). With high quality forage, increasing levels of supplemental grain may result in a decreased intake of forage due to a substitution of grain DE for forage DE. With low quality forage, however, increasing grain intake may have little effect on forage intake and grain DE and forage DE are additive.

Crude protein is often the main limiting nutrient for livestock in the tropics with approximately 7% as the minimum level required for positive nitrogen balance in mature grazing animals (Milford and Haydock, 1965). In ruminating cattle, the amino acids required may be obtained from dietary protein and some non-protein compounds. Protein is required for maintenance, growth, reproduction and lactation. Protein is especially important for the lactating cow because milk solids contain about 27% protein. Cattle store some protein in the blood, liver and muscle. These reserves may be used over a short-term period of protein deficiency, especially to maintain gestation and lactation (NRC, 1978). Irregular or delayed estrus is the major sign of protein shortage in diets for breeding females. Little (1975), in Australia, studied the effect of supplemental protein-P and P alone on pregnant cows grazing native pastures during the late dry season. The results showed that protein plus

P supplementation reduced greatly the interval from calving to first postpartum estrus. During the wet season in tropical areas, livestock gain weight rapidly since energy and protein supplies are adequate and thus the mineral requirements are high (McDowell, 1976). Van Niekerk (1974) reported that the beneficial effect of P was primarily during the wet season, although the P content in the grass was at its highest.

In the ruminant, the addition of fermentable carbohydrate to the diet increases the digestibility of dietary Mg. Also, absorption of Mg occurs mainly before reaching the duodenum. Volatile fatty acids (VFA) are the principal end products of microbial digestion of carbohydrate in the rumen and its absorption appears to have a striking influence on the transport of water and minerals, including Mg. Addition of lactose resulted in a decrease in acetate and an increase in propionate; rumen ammonia also decreased to a very low level (Rayssiguier and Poncet, 1980). The authors suggested that extra fermentable carbohydrates might be beneficial both in increasing absorption of Mg, Ca and P, in addition to energy.

Byers and Moxon (1980) conducted a study with Hereford steers to assess the relationship between Se adequacy and protein requirements to growing and finishing cattle. They found that Se levels are most critical during early stages of growth and when cattle are fed diets marginal or deficient in protein. Zinc deficiency also is reported to be a prominent feature associated with severe protein-energy malnutrition (PEM) (Underwood, 1981). Osteoporosis has been demonstrated in experimental animals fed a high protein diet. Also, it has been well established that increased consumption of protein in humans and animals results in increased urinary Ca excretion. Alkaline and acid phosphatase activity

in bone increased 2.5 and 2.3 times, respectively, reflecting increased matrix turnover induced by the high protein availability (Weiss et al., 1981).

Maternal-Fetal Relationships of Trace Elements in Ruminants

Many studies have demonstrated a variety of alterations in the fetus and in newborns when excesses or deficiencies of several mineral elements were offered to pregnant and lactating animals. Only in the past two decades have techniques for studying transfer and metabolism in vitro been defined (Hidiroglou and Knipfel, 1981). Trace elements enter the body of the fetus from very early in gestation. In order to do this, they must cross the placenta but how they achieve this is not entirely clear. Many of them circulate in the serum combined with protein in the fetal serum. The fetus is completely dependent upon the dam for its supply of minerals. Different types of placenta have shown varying degrees of permeability to minerals, carbohydrates, fats and proteins. In species in which placental barriers are strong, such as swine and ruminants, the fetus is partly supplied by absorption with the fetal placenta of the secretion from the uterine glands, i.e., the embryotropic route (Palludan et al., 1969).

In cattle, effects of Cu deficiency usually are postnatal while in sheep and goats, symptoms of Cu deficiency often occur in utero (Hidiroglou and Knipfel, 1981). High Cu content in most newborn animals has suggested placental transfer and storage before birth (Prior, 1964). However, little or no quantitive tissue studies on Cu placental transfer have been reported for ruminant animals. The daily amounts of Cu deposited in the total products of conception of the ewe during the first, second and third trimester averaged 15, 85 and 186 mg/day,

respectively (Moss et al., 1974). These data indicate a concentration barrier for Cu between the ewe and the fetus in the syndesmochorial type placenta. Lesions in the brain and spinal chord characteristic of enzootic ataxia could be detected as early as 99 days post-conception in fetal lambs where enzootic ataxia occurred (Smith et al., 1977). Seaman and Hartley (1981) studied congenital Cu deficiency in goats. They found Cu levels in the serum of kids and their dams and in the livers of the kids below normal. Williams and Bremner (1976) reported that Cu concentrations in liver increased towards the end of the gestation in ewes; liver Cu begins to decrease soon after birth, presumably from mobilization to meet the needs of other tissues of the growing animal. The pregnant ewe appears to be equipped poorly to protect her lamb against the effect of a dietary deficiency of Cu (Hidiroglou and Knipfel, 1981).

The mammalian newborn does not consistently carry higher total body Zn concentrations than mature animals of the same species (Underwood, 1977). There is a little fetal Zn storage; lactation represents a major homeostatic demand for Zn (Stake et al., 1975). In mature cows, homeostatic control mechanisms which regulate the Zn content of tissue are much more effective than in calves. Liver Zn concentration, initially very high, declined rapidly as pregnancy advanced (Williams and Bremner, 1976). An adequate Zn intake for gestation in the goat resulted in severe deficiency during lactation. Zinc is retained during pregnancy primarily in the placenta; transport varies with gestation, age and fetal placenta exchanged Zn with blood plasma four times faster than maternal placenta (NRC, 1979).

Studies in ruminants have indicated that Mn deficiency during gestation has deleterious effects on the developing embryo. Inadequate

dietary Mn induces an abnormal development of the epiphysical fetal cartilage (Hidiroglou and Knipfel, 1981). The concentration of Mn in the liver of newborn lambs appears to be useful for assessing the Mn status of the dam (Hidiroglou, 1979). Data presented by Hansard (1972, cited by Hidirouglou and Knipfel, 1981) suggest that Mn was transferred readily and comparatively rapidly from ewe to fetus. Following administration of ^{54}Mn to pregnant ewes, the concentration of radioactivity in the placenta peaked at 12 h post-injection, with the placental concentration representing more than 50% of the total ^{54}Mn concentration in the fetal compartment; after 168 h, more than half of the ^{54}Mn had accumulated in the fetus, with placental concentration decreasing to about 25% of the total fetal compartment. These data suggest that Mn was transferred rapidly from ewe to fetus. Neonatal calves born to dams on low Mn diets exhibited reduced Mn in liver and kidney (Rojas et al., 1965).

Perry et al. (1978) studied the effect of supplemental Se (0, 1, 2 or 5 mg per cow daily starting 90 days prepartum and extending through 6 to 7 months of lactation) on Se levels in blood serum of cows and their calves. The authors found that 5 mg/day levels increased calf serum Se over that of calves whose dams were fed the 1 and 2 mg levels. Also, calf liver and kidney Se levels were higher ($P < .05$) for calves from 5 mg Se-supplemented cows than for those from the control group. These data indicated that dietary Se of the cows was able to cross the placental membrane.

CHAPTER III
EFFECT OF ENERGY AND PROTEIN ON CALCIUM, PHOSPHORUS AND MAGNESIUM RETENTION AND MINERAL STORAGE BY SHEEP

Introduction

A proper supply of all essential nutrients is required for maximum animal performance under any type of environment. Inadequate supply of any given nutrient may adversely affect animal performance in different ways. Dietary requirements for minerals are more difficult to accurately define than those for the organic nutrients (energy and protein) because many factors affect mineral utilization.

A major problem for ruminant animals dependent on tropical grasslands is the prolonged dry season which lasts four to six months. During this period, grasses mature and become dry; consequently, the nutritional value of native pastures is low in protein, energy, vitamin A and specific minerals, particularly P. Animals gain weight during the rainy season when there is a plentiful supply of high quality forage which provides adequate protein and digesitble energy (Mtimuni, 1982). As forage quality declines during the dry season, animals may lose as much as 30% of their peak weight gained during the rainy season (Van Niekerk, 1974).

Insufficient energy probably limits performance of sheep more than other nutritional deficiencies and may result from inadequate amounts of feed or from feed of low quality. Poorly digested low-quality forages also lead to reduced feed intake. Forage may also be so high in water that energy intake is limited (N.R.C., 1975).

Insufficient protein intake also results in reduced appetite, lowered feed intake and lowered feed efficiency. Minson (1971) estimated the critical level of crude protein in pasture to be between 6.0 and 8.5% while Van Niekerk (1974) states that the major limiting nutrient from pasture is low protein content of grasses which usually falls below 7%. Protein supplementation without concurrent energy supplementation did not show response for beef cattle grazing natural pastures during the dry season in Zambia, Africa (Walker, 1957).

Energy and protein are closely related with the metabolism of Ca, P and Mg in the animal body. The principal objective of this experiment was to investigate the effects of two levels of energy-protein (low and high) and two levels of minerals (low and high) on Ca, P and Mg retention by the animal, and the effects of diets on mineral storage, blood parameters and mineral composition of selected animal tissues.

Experimental Procedure

Twelve Florida native crossbred wether lambs averaging 50 kg initial weight were randomly assigned to two treatment groups, where two levels of energy and protein and two levels of minerals were studied as they affected Ca, P and Mg retention.

In the first trial, the animals were fed a semi-purified diet (table 1) high in minerals (2 to 30 times maintenance requirements) with two levels of energy-protein (low = .8 x maintenance; high = 1.8 x maintenance). The animals were fed the two experimental diets for three months, housed in two pens (6 animals per pen) where water was available ad libitum, before being placed in metabolism cages for

TABLE 1. COMPOSITION OF EXPERIMENTAL DIETS

Ingredient (%)	IRN	Low Minerals		High Minerals	
		Diet 1	Diet 3	Diet 2	Diet 4
		Low energy Low protein	High energy High protein	Low energy Low protein	High energy High protein
Cottonseed hulls	1-01-599	38.00	8.00	38.00	8.00
Corn cobs	1-02-782	35.00	7.00	35.00	7.00
Starch (corn)	4-02-889	6.77	8.77	3.24	5.24
Soybean meal	5-04-604	5.50	10.50	5.50	10.50
Corn meal	4-02-861	7.00	60.00	7.00	60.00
Urea		1.00	1.00	1.00	1.00
Cerelose		3.00		3.00	
Corn oil	4-07-882	2.00	3.00	2.00	3.00
Mineral mix[a]		1.73	1.73	5.26	5.23
Vitamins A and D[b]		+	+	+	+
		100.00	100.00	100.00	100.00
Digestible energy, Mcal/ kg		2.48	3.40	2.35	3.28
Crude protein, %		8.30	13.45	8.30	13.45

[a] Supplied (g/100 kg) in diets 1 and 3 (low minerals) and diets 2 and 4 (high minerals), respectively: Sodium sulfate (Na_2SO_4) - 496.24, 1488.72; monosodium phosphate (H_2NaPO_4) - 495.94, 1487.82; calcium carbonate ($CaCO_3$) - 419.58, 1258.74; potassium sulfate (K_2SO_4) - 222.87, 668.61; sodium chloride (NaCl) - 48.49, 145.47; magnesium oxide (MgO) - 33.16, 99.47; ferrous sulfate ($FeSO_47H_2O$) - 4.98, 49.8; zinc carbonate ($ZnCO_3$) - 3.836, 38.358; manganese carbonate ($MnCO_3$) - 2.093, 20.925; sodium fluoride (NaF) - .442, 2.210; copper chloride ($CuCl_2$) - .423, 1.692; sodium molybdate ($Na_2MoO_42H_2O$) - .202, 2.018; sodium selenite (Na_2O_3Se) - .018, .350; potassium iodate (KIO_3) - .014, .135; cobaltus carbonate ($CoCO_3$) - .011, .101; Total - 1728.295, 5264.427.

[b] Supplied/kg of diet: 1020 IU Vitamin A and 222.4 IU Vitamin D_3 (diets 1 and 3); 12750 IU Vitamin A and 2780 IU Vitamin D_3 (diets 2 and 4).

retention studies. This balance trial consisted of an 8-d adjustment period and a subsequent 8-d total collection period (feces and urine).

In the second trial, dietary mineral concentrations were reduced and the same animals were fed again for another three months with a semi-purified diet (table 1), low in minerals with two levels of energy-protein (high and low) and housed in the same pens as previously. Following the 3-month period, animals were placed in individual metabolism cages for a retention study. At the end of this second trial, the lambs were sacrificed by exsanguination and liver, spleen, kidney, heart, muscle (longissimus dorsi) and metacarpal bones were removed and weighed and frozen for subsequent analysis.

Lambs were initially weighed, wormed (Levamisole) and allowed to adjust to experimental diets in metabolism cages for 8 days. The two balance trials involved an 8-d preliminary period followed by an 8-d total fecal and urinary collection period in which a constant daily feed intake of 1000 g per head was maintained. Water was supplied ad libitum. The rations were fed in the morning. During the 8-d collection period, urine was collected every 24 hours. Feces were collected each day into collection pans and aliquots of 10% of the total weight taken. Aliquots per period (24 hours) per lamb were mixed and stored at 0° C. Ration samples were collected each day in paper bags and at the end of the experiment were ground into 1 mm particle size in a Wiley mill. Feces were dried for 48 hours in a force-draft oven at 60° C. The dry fecal material was weighed and ground to pass a 1 mm sieve. Fecal and ration samples were analyzed for Ca, P and Mg using methods described by Fick et al., 1979.

All lambs were weighed monthly and blood samples collected at the end of each trial. Feed consumption, orts, fecal and urinary excretions and animal performance was recorded daily. Blood serum samples were deproteinized with 10% trichloracetic acid (TCA) and then analyzed for mineral content according to methods described by Fick et al. (1979). Calcium, Mg, Fe, Cu, Zn and Mn were analyzed by atomic absorption spectrophotometry (Perkin-Elmer 306) according to procedures recommended by the manufacturers (Anonymous, 1973). Phosphorus was determined by the colorimetric technique described by Harris and Popat (1954). Hemoglobin (Hb) was determined by the modification of the colorimetric method of Martinek (1970) and hematocrit by the microhematocrit method. Molybdenum concentrations were analyzed by flameless atomic absorption spectrophotometry using a Perkin-Elmer 503 according to procedures recommended by the manufacturer (Anonymous, 1974).

Ration and tissue samples were processed and analyzed for mineral content according to methods described by Fick et al. (1979). Liver and bone biopsies were taken at the end of each trial for mineral analysis. The sampling for liver biopsy was carried out as described by Fick et al. (1979) and bone biopsy in sheep as described by Little (1972).

Serum, wool and tissue Se were determined by a modification of the fluorimetric method (Whetter and Ullrey, 1978). Wool and dietary crude protein determination has been described by Technicon Industrial Systems (1978).

Specific gravity was determined for bone samples according to the procedure of Little (1972) as modified by Mtimuni (1982) and was

conducted as follows: Air-dried bone was weighed in air on an analytical balance. A soft piece of wire about 15 cm long with a noose at one end was weighed in air and later in water kept at 4° C in a 200 ml beaker. The wire was suspended from the balance by a hook and immersed into the water at the same level samples were to be immersed in water. The samples were dried and ether extracted following procedures outlined by Fick et al. (1979) and subsequently analyzed for Ca, P and Mg.

The biological utilization of Ca, P and Mg was measured as apparent absorption (total intake - fecal) and net retention (total intake - (fecal + urinary)). For example:

$$\text{Apparent Mg absorption} = \frac{\text{(Total Mg intake) - (Total fecal Mg)}}{\text{(Total Mg intake)}} \times 100$$

Net Mg Retention = (Total Mg intake) - (Total fecal Mg - Total urinary Mg)

Data were analyzed by the General Linear Model procedure of the Statistical Analysis System (Barr et al., 1976) utilizing the facilities of the Northeast Regional Data Center located on the campus of the University of Florida, Gainesville. Means and standard deviations were calculated; t-test was applied to find the significant difference among diets.

Results and Discussion

Body Weight and Blood Parameters

Daily intake of trace minerals in wether lambs are shown in table 2. Effect of energy-protein treatments on body weight, serum minerals, hemoglobin and hematocrit of lambs are presented in table 3. In

TABLE 2. DAILY INTAKE OF TRACE MINERALS

EXPERIMENT I, TRIALS 1 AND 2 *

Item	TRIAL 1 (HM)		TRIAL 2 (LM)	
	Diet 2[b] LEP	Diet 4[d] HEP	Diet 1[a] LEP	Diet 3[c] HEP
Fe, ppm	137	139	72	103
Cu, ppm	12	13	6	7
Zn, ppm	235	281	81	65
Mn, ppm	80	84	26	19
Co, ppm	1.2	1.0	.4	.8
Se, ppm	.19	1.2	.12	.51

* Daily intake of trace minerals based on feed intake and composition of experimental diets.

[a] Low mineral + low energy-protein.

[b] High mineral + low energy-protein.

[c] Low mineral + high energy-protein.

[d] High mineral + high energy-protein.

TABLE 3. EFFECT OF ENERGY- PROTEIN ON BODY WEIGHT, SERUM MINERALS, HEMOGLOBIN AND HEMATOCRIT IN SHEEP

EXPERIMENT I, TRIALS 1 AND 2[a]

	TRIAL 1				TRIAL 2			
	DIET 2		DIET 4		DIET 1		DIET 3	
Item	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1st wt. (kg)	47.80	12.40	43.10	11.60	49.70	15.40	59.00	18.00
2nd wt. (kg)	49.00	13.30	47.90	13.10	50.90[c]	15.30	64.10[d]	19.10
3rd wt. (kg)	49.50	16.20	51.00	15.10	50.40[c]	14.10	65.40[d]	21.60
4th wt. (kg)	47.60	11.90	52.70	17.90	-	-	-	-
Ca mg/100 ml	13.30	2.29	12.50	1.71	13.10	0.90	11.80	1.32
P mg/100 ml	5.70[e]	0.86	8.50[f]	1.30	6.20	1.32	7.20	0.81
Mg mg/100 ml	3.08	0.52	3.60	1.00	3.07	0.44	3.60	0.94
Na µg/ml	3057.70	117.88	3165.30	97.00	3057.70	111.01	3028.80	117.30
K µg/ml	169.20	16.63	198.50	42.00	161.30	17.96	167.20	16.32
Fe µg/ml	2.09	0.43	2.40	0.51	1.91	0.57	2.49	0.40
Cu µg/ml	0.97	0.41	1.20	0.19	0.82	0.14	0.85	0.27
Zn µg/ml	1.10	0.24	1.30	0.18	1.69	1.05	1.59	0.16

TABLE 3—CONTINUED

	TRIAL 1				TRIAL 2			
	DIET 2		DIET 4		DIET 1		DIET 3	
Item	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Se µg/ml	0.16[e]	0.01	0.21[f]	0.02	0.118	0.014	0.130	0.024
Hemoglobin g/ 100 ml	11.67	0.65	10.58	1.44	11.92	1.51	12.92	1.75
Hematocrit, %	53.00	4.29	45.75	9.00	50.00	6.96	51.80	7.26

[a] Means based on six observations on diet 2 and four on diet 4.

[b] Means based on six observations on diet 1 and five on diet 3.

[c,d] Means in the same row with different superscripts differ ($P < .05$).

[e,f] Means in the same row with different superscripts differ ($P < .01$).

trial 1, no differences ($P > .05$) were found between diets 2 and 4 (high in minerals, with low and high energy-protein, respectively) in relation to monthly gain (kg). In trial 2, differences ($P < .05$) were found in lambs fed diets 1 and 3 (low in minerals) in both the second and third weighings (50.90 vs 64.10 kg and 50.50 vs 65.50 kg, respectively). Serum P and Se were higher ($P < .01$) in diet 4 (HEP + HM) versus diet 2 (LEP + HM): 8.5 vs 5.6 mg/100 ml P and 0.21 vs 0.16 µg/ml Se, respectively. No differences in serum Ca, Mg, Na, K, Fe, Cu, Zn, Hb and hematocrit were found ($P < .05$) as a result of different energy-protein concentrations between diets 2 and 4. There were also no differences in serum minerals, Hb and hematocrit concentrations for animals fed low minerals with different energy-protein concentrations (diets 1 and 3). Paired comparisons between diets 1 and 2 (LEP + LM vs LEP + HM) and 3 and 4 (HEP + LM vs HEP + HM) were made for hematocrit, Hb and serum minerals with no differences found ($P > .05$).

Wethers fed high energy-protein diets with either high or low mineral concentrations had greater gains than diets low in energy-protein. During this 7-month experiment, animals fed low energy-protein diets with high and low minerals increased only 2.60 kg body weight (47.8 kg initial weight vs 50.4 final weight), as compared with animals fed high energy-protein diets with high and low minerals which increased 22.3 kg body weight (43.10 kg initial weight vs 65.40 kg final weight). High energy-protein diets (1.8 x maintenance requirements) increased wethers' average daily gain 106.2 g/animal, while wethers fed low energy and protein diets (.8 x maintenance requirements) averaged only 12.4 g/d/animal.

Rosa (1980) studied the P, Al and Fe interrelationships in sheep and reported that the addition of 0.25% P to the basal diet improved ($P < .01$) average daily gain from 105 to 148 g/animal; also, excess of dietary Fe (800 ppm) decreased ($P < .01$) average daily gain from 165 to 97 g/animal.

The high energy-protein diet with high minerals (diet 4) increased only serum P and Se. Rosa (1980) found that excess dietary Fe (800 ppm) increased ($P < .01$) inorganic serum P (6.3 to 7.6 mg/100 ml). This author also found that blood Hb and hematocrit levels increased when dietary Fe was increased (11.4 to 16.3 g/100 ml Hb and 33.9 to 46.6% hematocrit, respectively).

Levels of energy-protein and increased levels of dietary minerals did not affect serum mineral concentrations; it seems through homeostatic mechanisms that the animal mobilizes minerals from body reserves to maintain normal serum concentrations.

Liver Minerals

None of the six minerals analyzed in liver (table 4) in trial 1 were affected ($P > .05$) by dietary treatment. In trial 2, however, the animals receiving low mineral diets with high energy-protein (diet 3) had lower ($P < .05$) concentrations of Fe, Cu and Co with no differences ($P > .05$) found in Zn, Mn, Mo and Se concentrations. High dietary energy-protein concentrations in the presence of low minerals apparently depressed liver Fe, Cu and Co.

Paired comparisons between diets 1 and 2 (LEP + LM vs LEP + HM) and 3 and 4 (HEP + LM and HEP + HM) were also made which would include both time and treatment effects. No differences ($P > .05$) were found

TABLE 4. EFFECT OF ENERGY-PROTEIN ON LIVER MINERAL COMPOSITION IN SHEEP

EXPERIMENT I, TRIALS 1[a] AND 2[b]

	TRIAL 1				TRIAL 2			
	DIET 2		DIET 4		DIET 1		DIET 3	
Mineral DM Basis	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fe, ppm	356.17	93.24	496.67	425.80	339.00[c]	170.95	150.80[d]	49.89
Cu, ppm	319.33	326.10	201.00	124.10	214.67[c]	117.23	68.20[d]	36.60
Zn, ppm	241.00	179.86	198.67	180.10	79.00	14.17	83.00	17.50
Mn, ppm	9.97	4.16	7.31	1.07	4.38	1.29	5.90	2.62
Co, ppm	0.59	0.34	0.54	0.33	0.24[c]	0.05	0.17[d]	0.021
Mo, ppm	5.67	1.75	3.74	1.23	3.97	1.18	4.14	0.67
Se, ppm	-	-	-	-	0.60	0.24	0.84	0.30

[a] Means based on six observations on diet 2 and four on diet 4.

[b] Means based on six observations on diet 1 and five on diet 3.

[c,d] Means in the same row with different superscripts differ ($P < .05$).

in Fe, Cu, Zn, Mn, Co and Mo liver concentrations between diets 3 and 4. However, liver Mn and Co concentrations were higher ($P < .05$) for diet 1 vs 2, respectively, as follows: 9.97 vs 4.38 ppm Mn, and 0.59 vs 0.24 ppm Co. Wethers fed diet 2 (LEP + HM) also had higher ($P < .1$) liver Zn concentrations than animals fed diet 1 (LEP + LM) (241.0 vs 79.0 ppm, respectively).

Wethers fed high mineral diets had higher concentrations of liver Mn, Co and Zn. Obviously reliable comparisons cannot be made between two trials because of the time difference but trends can be observed. Mean liver Fe concentrations in wethers fed the four experimental diets were as follows: 497 ppm, Diet 4; 356 ppm, Diet 2; 339 ppm, Diet 1; and 151 ppm, Diet 3. McDowell et al. (1980) reported the critical Fe levels to be 180 ppm in cattle. Based on this critical level, wether mean liver Fe concentrations were adequate except in animals fed diet 3 (HEP + LM) in which they were below the critical level. Ammerman et al. (1967) found mean liver Fe concentrations of 169 ppm in calves fed Fe-deficient diets.

Liver Cu is reported to be the best criterion for assessing the Cu status of cattle (CMN, 1973). McDowell et al. (1980) reported the critical level for Cu to be between 25 and 75 ppm in cattle. In the four experimental diets, wether liver Cu concentrations are higher than these critical levels. Miller and Miller (1962) reported a range of 84 to 132 ppm liver Zn concentrations for apparently healthy cattle. Although not significant ($P > .03$), high mineral diets tended to increase liver Zn concentrations in animals (241.0 and 198.7 vs 79.0 and 83.0 ppm for diets 2, 4, 1 and 3, respectively). Liver Mn concentrations were above the critical concentration of 6 ppm

(McDowell and Conrad, 1977) in both diets high in minerals (9.97 and 7.31 ppm in diets 2 and 4, respectively) but below the critical concentration in both diets low in minerals (diets 1 and 3, 4.38 and 5.90 ppm, respectively).

Mean liver Co concentrations were higher in trial 1 in both diets high in minerals (diets 2 and 4, .59 and .54 ppm, respectively) when compared to diets low in minerals (diets 1 and 3, .24 and .17 ppm) of trial 2. Despite this difference, none of the liver samples contained levels below .05 ppm Co cited by McDowell and Conrad (1977) as borderline to deficient for grazing cattle.

Lebdosoekojo (1977) reported liver Mo levels in young bulls on native pastures with and without mineral supplementation as 3.9 and 4.5 ppm, respectively. In the present experiment for treatments 2, 3, 1 and 4, liver Mo concentrations were 5.67, 4.14, 3.97 and 3.74 ppm, respectively. All animals in the second trial (diets 1 and 3) contained liver Se levels above the .25 ppm reported by McDowell et al. (1978) as normal for grazing cattle.

Kidney, Heart, Spleen and Muscle Minerals

Results indicating the effects of energy-protein on tissue mineral composition (kidney, heart, spleen and muscle) at the end of trial 2 (low minerals) are presented in table 5. The low energy-protein diet increased ($P < .05$) kidney Fe concentration compared to the high energy-protein diet (641.0 vs 165.2 ppm, respectively). No differences ($P > .05$) were found for any other tissue mineral.

In investigations with ruminants, Standish et al. (1969, 1971) and Standish and Ammerman (1971) consistently observed an elevation

TABLE 5. EFFECT OF ENERGY-PROTEIN ON TISSUE MINERAL COMPOSITION IN SHEEP

EXPERIMENT I, TRIAL 2[a]

	KIDNEY				HEART				SPLEEN				MUSCLE			
	DIET 1		DIET 3		DIET 1		DIET 3		DIET 1		DIET 3		DIET 1		DIET 3	
DM BASIS	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fe, ppm	641.00[b]	333.00	165.2[c]	72.4	160.70	79.0	154.2	32.80	546.5	295.90	630.00	343.50	79.30	13.30	70.40	16.80
Cu, ppm	18.17	11.70	15.80	5.80	15.00	7.65	16.80	3.20	9.33	4.97	6.50	1.29	10.00	7.40	13.20	8.90
Zn, ppm	72.0	17.20	67.40	12.10	55.70	16.90	58.00	10.30	112.67	114.00	92.50	14.18	87.67	18.80	121.00	52.70
Mn, ppm	2.39	1.07	2.20	0.64	0.84	0.26	1.08	0.32	0.83	0.46	0.89	0.15	1.50	0.99	1.23	0.93
Co, ppm	0.16	0.05	0.17	0.07	0.068	0.04	0.08	0.04	0.06	0.04	0.08	0.03	0.03	0.03	0.17	0.35
Mo, ppm	2.74	1.13	2.44	0.15	0.21	0.08	0.20	0.03	0.34	0.16	0.33	0.05	0.08	0.03	0.07	0.03
Se, ppm	-	-	-	-	-	-	1.15	0.04	-	-	-	-	-	-	-	-

[a] Means based on six observations on diet 2, and five on diet 4 (except Se).

[b,c] Means in the same row with different superscripts differ ($P < .05$).

of Fe content in liver, spleen, kidney, heart and muscle when excess dietary Fe was supplied to steers or sheep. Rosa (1980) reported that high dietary P or Fe decreased Cu concentration in kidney. Also, Cu was depressed in spleen by diets high in P. Similar results were observed by Talliris (1977), who reported a reduction in kidney Cu by increasing dietary P.

In the present experiment, the levels of energy-protein in the presence of low mineral diets did not affect mineral concentrations in these tissues; only Fe was higher ($P < .05$) in kidneys of wethers fed diet 1 (LE - LM). With these data, we assumed that the period of time (3 months) was not enough to deplete the animals and we only have data for tissue mineral concentrations of kidney, heart, spleen and muscle at the end of trial 1 (low mineral diets) when animals were slaughtered.

Wool Nitrogen and Mineral Concentrations

Effects of energy-protein treatments on wool nitrogen and mineral concentrations are presented in Table 6 (trial 1). No difference ($P > .05$) was found between diets 1 and 3 in nitrogen. The diets high in energy-protein with low minerals was higher ($P < .05$) in wool P and Na (171 vs 117 ppm and 1141.00 vs 730.03 ppm, respectively); Mg also was higher ($P < .01$) (68 vs 43 ppm in diets 3 and 1, respectively). No differences ($P > .05$) were found between diets 1 and 3 in wool Ca, K, Fe, Cu, Zn, Mn, Mo and Se concentrations. Diet 1 tended to be higher in those elements except in Mn but this trend was not significant ($P > .05$).

TABLE 6. EFFECT OF ENERGY-PROTEIN ON WOOL NITROGEN AND MINERAL CONCENTRATIONS IN SHEEP

EXPERIMENT I, TRIAL 2[a]

	Diet 1		Diet 3	
	Mean	SD	Mean	SD
N, %	14.27	0.14	14.19	0.14
Ca, ppm	139.00	20.51	154.60	25.30
P, ppm	117.17[b]	33.88	171.40[c]	20.89
Mg, ppm	42.83[d]	14.80	68.40[f]	7.70
Na, ppm	795.83[b]	273.81	1145.80[c]	220.35
K, ppm	3444.67	927.35	4700.40	1578.86
Fe, ppm	9.86	2.32	10.80	3.24
Cu, ppm	4.32	0.66	4.37	1.28
Zn, ppm	841.50	110.52	995.40	150.56
Mn, ppm	0.356	0.051	0.340	0.087
Mo, ppm	0.084	0.080	0.077	0.050
Se, ppm	0.391	0.076	0.344	0.081

[a] Means based on six and five observations on diets 1 and 3, respectively.

[b,c] Means in the same row with different superscripts differ ($P < .05$).

[d,f] Means in the same row with different superscripts differ ($P < .01$).

There has been considerable interest recently in the use of hair as an indicator of the mineral status of animals. In contrast to liver and other tissues, hair is an easy biological material to collect and is stable. Trace elements, in particular, are accumulated in hair in concentrations that are generally at least ten times higher than those present in blood serum or urine and may provide a continuous record of nutritional status and exposure to heavy metal pollutants (Maugh, 1978). Hair acts as a recording filament because elements are deposited in the hair matrix within a short time and are removed from active metabolism as the hair shaft grows from the follicle. The major disadvantage of using hair as a biopsy material is that many factors other than diet are known to affect mineral content of hair. Factors that have significant effects on mineral concentrations in hair include season, breed, age, hair color and body location (Combs et al., 1982). As an example, Cu deficiency in ruminants is often associated with depigmentation and impaired keratinization of hair. Copper content of hair from mammals has been studied as a potential index of Cu status. O'Mary et al. (1970) reported that level of dietary Cu affected concentration of Cu in hair of Holstein and Hereford cattle. White hair from both breeds was affected more than pigmented hair, and Cu content of black Holstein hair was not consistent with increasing levels of dietary Cu.

In the present experiment, high levels of energy-protein with low minerals (diet 3) increased only P, Mg and Na concentrations in wool; the other mineral elements were within the normal range.

Bone Mineral Composition

Rib mineral concentrations

Effects of energy-protein treatments on rib mineral biopsy concentrations are presented in table 7. For trial 1, differences ($P < .05$) were found between diets 2 and 4 (high minerals) only in Mg, expressed both as % dry fat-free bone (.59 vs .71%, respectively) and as % ash (.97 vs 1.185), being higher in each case for the high energy-protein diet. In trial 2, Mg expressed as % bone ash was higher ($P < .01$) in diet 3 (HEP + LM) than diet 1 (LEP + LM) (1.16 vs 0.89%, respectively). Specific gravity (g/cc) was higher ($P < .05$) in rib bone of wethers fed diet 1 than those fed diet 3 (1.53 vs 1.39 g/cc, respectively). Calcium and P concentrations in bone were not affected ($P > .05$) between diets 2 and 4 of trial 1 and diets 1 and 3 of trial 2. No differences ($P > .05$) were found in bone parameters and treatment effects between diets 1 and 2, and 3 and 4, which included both time and treatment effects.

Means for rib bone Ca in wethers fed the four experimental diets were below the critical level of 24.5% considered normal for cattle (Little, 1972). Bone P has been indicated as the best estimate of P status for grazing ruminants compared to blood and hair P (Cohen, 1973). Means for rib bone P were also below the critical level of 11.5% P for normal cattle (Little, 1972). Unlike serum P, bone P, because of slow mobilization from the bones, is reported not to be influenced by rapid changes in diet, exercise, excitement or when laboratory facilities preclude immediate analysis of samples (Cohen, 1973).

TABLE 7. EFFECT OF ENERGY-PROTEIN ON BONE RIB MINERAL CONCENTRATIONS IN SHEEP EXPERIMENT I, TRIALS 1[a] AND 2[b]

	TRIAL 1				TRIAL 2			
	DIET 2		DIET 4		DIET 1		DIET 3	
Parameter	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Bone (DM Fat Free)								
Ca, %	20.38	0.98	19.35	1.72	19.57	2.97	21.40	3.73
P, %	8.73	2.75	8.50	3.02	9.75	2.32	11.46	2.32
Mg, %	0.59[c]	0.06	0.71[d]	0.10	0.55	0.09	0.70	0.17
Ash, %	60.65	0.89	60.18	0.78	61.03	1.17	59.68	9.52
Ca, % Ash	33.60	1.56	32.15	2.80	32.03	4.86	35.80	1.17
P, % Ash	14.42	4.50	14.15	5.16	15.98	3.77	19.14	1.54
Mg, % Ash	0.97[c]	0.097	1.18[d]	0.19	0.89[e]	0.13	1.16[f]	0.12
Specific gravity gm/cc	1.57	0.10	1.49	0.03	1.53[c]	0.09	1.39[d]	0.10
Ca, mg/cc	321.50	24.79	286.25	28.25	297.17	45.32	297.80	53.08
P, mg/cc	135.33	35.02	126.25	47.27	148.50	37.11	159.00	30.94
Mg, mg/cc	9.20	0.90	10.58	1.78	8.30	1.41	9.74	2.24
Ca:P ratio	2.53	0.78	2.48	0.75	2.08	0.51	1.88	0.10

[a] Means based on six observations on diet 2 and four on diet 4.

[b] Means based on six observations on diet 1 and five on diet 3.

[c,d] Means in the same row with different superscripts differ ($P < .05$).

[e,f] Means in the same row with different superscripts differ ($P < .01$).

Magnesium, when expressed as % bone ash, was above the .60% bone ash Mg level found by Lebdosoekojo (1977) in healthy grazing young bulls. Based on a critical level of 66.8% (Little, 1972) for 11th rib ash content in cattle, wethers' rib bone ash were below this value for the four treatments.

Phosphorus intake has been reported to influence bone density or specific gravity (Little, 1972). In this study, the specific gravity of ribs was not altered by increased energy-protein and minerals in the experimental diets. Lebdosoekojo (1977) found that the age of the animal affected the density of the dry, fat-free metacarpal diaphyses. The density of 14-month-old bulls was greater ($P < .05$) than that of 24-month-old bulls. Means of rib bone Ca:P ratios are: 2.53, 2.48, 2.08 and 1.88 in wethers fed diets 2, 4, 1 and 3, respectively.

Metacarpal bone mineral concentrations

Effects of energy-protein treatments on metacarpal mineral concentrations are presented in table 7. Means based on six and five observations in diets 1 and 3, respectively, differed only in the second trial. No differences ($P > .05$) were found between the two experimental diets in any metacarpal bone parameters.

In wethers fed diets low in minerals (1 and 3), higher values of metacarpal bone mineral concentrations were found than in rib bone mineral concentrations. The expression of mineral concentration per unit of volume was considered better than expression in % of bone ash since the density and ash % are already taken into account (Lebdosoekojo, 1977). Little (1972) also reported that bone minerals

TABLE 8. EFFECT OF ENERGY-PROTEIN ON BONE METACARPAL MINERAL CONCENTRATIONS IN SHEEP

EXPERIMENT I, TRIAL 2[ab]

	DIET 1		DIET 3	
Parameter	Mean	SD	Mean	SD
Bone (DM Fat Free)				
Ca, %	22.650	0.812	22.700	1.382
P, %	13.417	0.422	13.400	0.914
Mg, %	0.492	0.051	0.534	0.073
Ash, %	68.067	1.33	67.340	2.223
Ca, % Ash	33.380	0.764	33.700	1.412
P, % Ash	19.633	0.427	19.880	0.965
Mg, % Ash	0.717	0.070	0.794	0.124
Specific Gravity gm/cc	1.790	0.121	1.742	0.132
Ca, mg/cc	409.17	43.47	396.40	49.89
P, mg/cc	257.00	48.76	234.00	30.66
Mg, mg/cc	8.80	1.252	9.28	1.110
Ca:P ratio	1.705	0.056	1.692	0.019

[a] Means based on six and five observations on diets 1 and 3, respectively

[b] No significant differences were found between treatments ($P > .05$).

expressed in unit of volume were more sensitive to detect nutrient status than when they were expressed in percent ash.

Blincoe et al. (1973) did not find the differences in concentrations of P and Ca in caudal vertebrae, rib and femur in range cattle.

Calcium, Phosphorus and Magnesium Retention

Calcium, P and Mg retention in trials 1 and 2 are shown in table 9. In trial 1 (high minerals), Ca and P retention was less ($P < .05$) with diets low in energy-protein (1.41 vs 2.74 g/d Ca and 2.57 vs 4.53 g/d P). Also, Mg retention was less ($P < .01$) in wethers fed the low energy-protein diet (1.32 vs 2.85 g/d). In trial 2, differences ($P < .01$) were found between diets 1 and 3 in Ca, P and Mg retention (.724 vs 1.505 g/d, 1.321 vs 2.773 g/d, and 1.124 vs .840 g/d, respectively). Table 10 shows the individual values of Ca retention in wethers fed four experimental diets. Also included in this table are values for average 8-day intake, fecal and urinary excretion, total excretion, apparent absorption, percent and Ca retention expressed as g/8-day and g/d. All lambs were in positive Ca balance during these two trials. Calcium fecal excretion was higher than urinary excretion in these two retention trials (98.2, 97.6, 90.7 and 94.8% Ca fecal excretion for diets 2, 4, 1 and 3, respectively).

Table 11 shows individual values of P retention. Fecal P excretion was higher than urinary P excretion, except diet 3 (97.8, 72.3, 89.4 and 38.4% P fecal excretion for diets 2, 4, 1 and 3, respectively). Diets 4 and 3 (high energy-protein with high and low minerals, respectively) tend to increase urinary P excretion more than diets 2 and

TABLE 9. EFFECT OF ENERGY-PROTEIN ON CALCIUM, PHOSPHORUS AND MAGNESIUM RETENTION IN SHEEP

EXPERIMENT I, TRIALS 1[a] AND 2[b]

CALCIUM RETENTION (G/DAY)

TRIAL 1				TRIAL 2			
Diet 2		Diet 4		Diet 1		Diet 3	
Mean	SD	Mean	SD	Mean	SD	Mean	SD
1.410[c]	0.798	2.737[d]	0.636	0.724[e]	0.280	1.505[f]	0.268

PHOSPHORUS RETENTION (G/DAY)

Mean	SD	Mean	SD	Mean	SD	Mean	SD
2.569[c]	0.335	4.538[d]	1.274	1.321[e]	0.310	2.773[f]	0.329

MAGNESIUM RETENTION (G/DAY)

Mean	SD	Mean	SD	Mean	SD	Mean	SD
1.317[e]	0.673	2.847[f]	0.753	1.124[e]	0.038	0.840[f]	0.113

[a] Means based on six observations on diet 2 and four on diet 4.

[b] Means based on six observations on diet 1 and five on diet 3.

[c,d] Means in the same row with different superscipts differ ($P < .05$).

TABLE 9 —CONTINUED

[e,f] Means in the same row with different superscripts differ ($P < .01$).

TABLE 10. EFFECT OF ENERGY-PROTEIN ON CALCIUM RETENTION

EXPERIMENT I, TRIALS 1 AND 2

Item	Wether No.	Intake g/8 days	CALCIUM EXCRETION			RETENTION	
			Fecal g/8 days	Urinary g/8 days	Total Excretion g/8 days	Apparent Absorption, %[a]	g/8 days	g/day
Trial 1	1	43.10	23.10	0.56	23.66	46.40	19.44	2.43
	2	44.70	35.80	0.40	36.20	19.90	8.50	1.06
	3	44.70	36.20	1.03	37.23	19.00	7.47	0.93
Diet 2	4	44.70	32.20	0.41	32.61	28.00	12.09	1.51
	5	45.50	27.50	0.44	27.94	39.60	17.56	2.20
	6	45.50	42.00	0.86	42.86	7.70	2.64	0.33
	7	52.60	31.00	0.21	31.21	41.10	21.39	2.67
Diet 4	8	51.40	22.50	1.23	23.73	56.20	27.67	3.46
	9	30.00	14.30	0.32	14.62	52.30	15.38	1.92
	10	47.00	23.50	0.35	23.85	50.00	23.15	2.90
Trial 2	1	17.10	9.20	0.61	9.81	46.20	7.29	0.91
	2	17.00	8.70	0.38	9.08	48.80	7.92	0.99
	3	17.20	11.60	3.40	15.00	32.60	2.20	0.28
Diet 1	4	17.20	8.90	0.74	9.64	48.30	7.56	0.95
	5	17.10	11.50	0.36	11.86	32.70	5.24	0.66
	6	17.20	12.00	0.68	12.68	30.20	4.52	0.56
	7	20.10	7.10	0.16	7.26	64.70	12.84	1.61
	8	17.60	7.70	0.26	7.96	56.30	9.64	1.21
Diet 3	9	21.00	7.70	0.48	8.18	63.30	12.82	1.60
	10	20.30	5.20	0.30	5.50	74.40	14.80	1.85
	11	21.80	10.90	0.81	11.71	50.00	10.09	1.26

[a] Apparent absorption, % = $\frac{\text{Intake - Fecal}}{\text{Intake}} \times 100$

TABLE 11. EFFECT OF ENERGY-PROTEIN ON PHOSPHORUS RETENTION

EXPERIMENT I, TRIALS 1 AND 2

	Wether No.	Intake g/8 days	PHOSPHORUS EXCRETION			RETENTION	
			Fecal g/8 days	Urinary g/8 days	Total Excretion g/8 days	Apparent Absorption, %[a]	g/8 days	g/day
Trial 1	1	37.10	19.60	0.053	19.65	47.20	19.45	2.18
	2	38.50	17.20	0.091	17.29	55.30	21.21	2.65
Diet 2	3	38.50	18.50	0.010	18.51	51.90	19.99	2.49
	4	38.50	19.80	0.394	20.19	48.60	18.31	2.29
	5	39.20	12.60	1.719	14.32	67.90	24.88	3.11
	6	39.20	17.50	0.115	17.62	55.40	21.58	2.70
	7	70.30	22.70	7.994	30.69	67.70	39.61	4.95
Diet 4	8	68.80	21.50	1.176	22.68	68.80	46.12	5.76
	9	40.10	6.60	11.540	18.14	83.50	21.96	2.75
	10	62.90	19.00	6.019	25.02	69.80	37.88	4.74
Trial 2	1	21.40	9.10	0.800	9.20	57.50	12.20	1.53
	2	21.20	5.90	2.300	8.20	72.20	13.00	1.63
	3	21.40	14.50	0.400	14.90	32.20	6.50	0.81
Diet 1	4	21.40	8.60	0.100	8.70	59.80	12.70	1.59
	5	21.40	7.00	3.600	10.60	67.30	10.80	1.35
	6	21.40	12.20	0.300	12.50	43.00	8.90	1.11
	7	36.40	4.50	10.200	14.70	87.60	21.70	2.71
	8	31.90	3.60	5.400	9.10	88.70	22.80	2.85
Diet 3	9	38.00	8.10	7.300	15.40	78.70	22.60	2.83
	10	36.70	4.10	14.400	18.50	88.80	18.20	2.28
	11	39.50	7.20	6.800	14.00	81.80	25.50	3.19

[a] Apparent Absorption, % = $\frac{\text{Intake} - \text{Fecal}}{\text{Intake}} \times 100$

1 (low energy-protein with high and low minerals, respectively). Table 12 shows individual values of Mg retention. All lambs were in positive Mg balance during the two trials, except one animal on diet 2. Magnesium urinary excretion was higher than fecal excretion (72.0, 77.3, 71.6 and 65.9% Mg urinary excretion for diets 2, 4, 1 and 3, respectively).

Table 13 also shows paired comparisons between diets 1 and 2, and 3 and 4 in Ca, P and Mg retention. Although these comparisons take into account both time and treatment aspects, a difference ($P < .1$) between diets 2 and 1 (1.41 vs .73 g/day) and diets 4 and 3 ($P < .05$) (2.74 vs 1.51 g/day) in Ca retention was observed. Animals fed diets 2 and 4 (LEP + HM and HEP + HM) had increased Ca retention, expressed in grams per day; also these animals had a higher ($P < .01$) P retention value (diet 2, 2.569 vs diet 1, 1.321 g/day, and diet 4, 4.538 vs diet 3, 2.773 g/day, respectively) ($P < .1$). In retention to Mg, differences ($P < .05$) between diets 4 and 3 (2.847 vs .840 g/day) were found. Diets with high mineral concentration tended to increase Ca, P and Mg retention in animals, independent of the level of energy and protein. Means for Ca retention were: 2.737, 1.505, 1.410 and .724 g/day for diets 4, 3, 2 and 1, respectively. Phosphorus retention means were: 4.538, 2.773, 2.569 and 1.321 g/day for diets 4, 2, 1 and 3, respectively.

Moore et al. (1972) used two levels (10 and 32% on dry basis) of crude protein (N) and two levels of nonprotein nitrogen (urea) to study Mg utilization and found no differences ($P < .05$) in apparent Mg absorption between the four rations, indicating that high ruminal fluid ammonia levels did not interfere with Mg absorption. These

TABLE 12. EFFECT OF ENERGY-PROTEIN ON MAGNESIUM RETENTION

EXPERIMENT I, TRIALS 1 AND 2

Item	Wether No.	Intake g/8 days	MAGNESIUM EXCRETION			RETENTION	
			Fecal g/8 days	Urinary g/8 days	Total Excretion g/8 days	Apparent Absorption, %[a]	g/8 days	g/day
Trial 1	1	22.60	2.40	5.90	8.30	89.40	14.30	1.79
	2	23.50	3.90	7.20	11.10	83.40	12.40	1.55
	3	23.50	3.70	7.20	10.90	84.30	12.60	1.58
Diet 2	4	23.50	3.60	7.20	10.80	84.70	12.70	1.59
	5	23.90	3.20	9.20	12.40	86.60	11.50	1.44
	6	23.90	5.00	19.20	24.20	79.10	-0.30	-0.04
	7	41.70	4.10	9.30	13.40	90.20	28.30	3.54
Diet 4	8	40.80	3.10	11.80	14.90	92.40	25.90	3.24
	9	23.80	1.60	7.70	9.30	93.30	14.50	1.81
	10	37.30	3.10	11.80	14.90	91.70	22.40	2.80
Trial 2	1	11.90	0.70	1.85	2.55	94.10	9.35	1.17
	2	11.80	0.71	1.80	2.51	94.00	9.29	1.16
	3	11.90	0.92	2.42	3.34	92.30	8.56	1.07
Diet 1	4	11.90	0.69	2.45	3.14	94.20	8.76	1.10
	5	11.90	0.98	1.90	2.88	91.80	9.02	1.13
	6	11.90	0.93	1.98	2.91	92.20	8.99	1.12
	7	11.10	1.47	3.89	5.36	86.80	5.74	0.72
	8	9.70	0.60	1.59	2.19	93.80	7.51	0.94
Diet 3	9	11.60	1.68	2.81	4.49	85.50	7.11	0.89
	10	11.20	1.66	3.79	5.45	85.20	5.75	0.72
	11	12.00	2.18	2.32	4.50	81.80	7.50	0.94

[a] Apparent absorption, $\% = \frac{\text{Intake} - \text{Fecal}}{\text{Intake}} \times 100$

TABLE 13. PAIRED COMPARISONS BETWEEN DIETS 2 VERSUS 1; AND 4 VERSUS 3 FOR BODY WEIGHT, Ca PERCENT IN RIB BONE ASH, Zn, Mn, AND Co IN LIVER AND Ca, P, AND Mg RETENTION IN WETHERS

	DIET 1 VS. DIET 2		DIET 3 VS. DIET 4	
Variable	Mean	Mean	Mean	Mean
Weight 1st (kg)	49.7	47.8	59.00**	43.10
Weight 2nd (kg)	50.9	47.0	64.10**	47.90
Weight 3rd (kg)	50.4	49.5	65.40**	51.00
Ca, % Ash rib bone	32.03	33.60	35.80+	32.15
Zn, µg/g liver	79.00	241.00+	83.00	198.67
Mn, µg/g liver	4.38	9.97*	5.90	7.31
Co, µg/g liver	0.24	0.59*	0.17	0.54
Ca, retention g/day	1.41+	0.724	1.505	2.737*
P, retention g/day	1.321	2.569**	2.773	4.538+
Mg, retention g/day	1.124	1.317	0.840	2.847*

+ Significant at ($P < .1$).

* Significant at ($P < .05$).

** Significant at ($P < .01$).

investigators also found urinary Mg excretion higher and retention lower ($P < .01$) for the animals consuming the high-nitrogen rations. Stilling et al. (1964) found that Mg excretion on low-N forages resulted in 82% excreted via feces and 13% via urine. Comparable values on high-N forages were 88% and 10.7%, respectively. Duton and Fontenot (1967), in contrast, found that 36 to 42% of total Mg was excreted via urine.

Rosero (1975) found that Ca retention was lower for the lambs fed the high N rations. These results differ in part with those of Stillings et al. (1964) who reported a higher Ca retention from feeding high N-fertilized forages. However, Moore et al. (1972) found that urinary Ca excretion was greater and retention was lower for the animals fed high-N rations.

In the present experiment, the major route for Ca and P excretion was feces and for Mg excretion, urine. Urinary Mg excretion was particularly higher in wethers fed diets which were high in minerals (diets 2 and 4) in comparison with wethers fed diets low in minerals (diets 1 and 3). According to the CMN (1973), daily urinary Mg excretion is a better criterion of Mg adequacy than plasma concentration. Homeostatis is maintained by the excretion of excess Mg via urine. Appendix table 38 shows chemical composition of experimental diets and appendix tables 39 through 44 summarizes the individual levels of tissue mineral concentrations.

Summary

Two trials were conducted to study the effects of different energy and protein levels on mineral utilization by sheep. Twelve Florida native crossbred wether lambs averaging 50 kg initial weight were

randomly assigned to two treatment groups. In the first trial, the animals were fed a semi-purified diet, high in minerals (HM) with either low-energy-protein (LEP) or high energy-protein (HEP) concentrations. Serum P and Se were higher ($P < .05$) in the HM + HEP versus HM + LEP diets, 8.53 vs 5.60 mg/100 ml and .21 vs .16 µg/ml, respectively. No differences ($P > .05$) were found between diets in liver mineral concentrations. Differences ($P < .05$) were found between diets HM + LEP and HM + HEP, in bone Mg concentration, expressed as percent dry, fat-free bone, .59 vs .71%, respectively. Also, bone Mg expressed as percent ash was higher ($P < .05$) with the increased energy-protein, .97 vs 1.18%.

Calcium and P retention were lower ($P < .05$) in the diet low in energy-protein than the diet high in these nutrients (1.41 vs 2.73 and 2.57 vs 4.53 g/d, respectively). Also, Mg retention was less ($P < .01$) in wethers fed diets low in energy-protein versus wethers fed diets high in energy-protein, 1.32 vs 2.85 g/d.

In the second trial, animals were fed a semi-purified diet low in minerals (LM) with either low-energy-protein (LEP) or high energy-protein (HEP) concentrations. The diet LM + LEP was higher ($P < .05$) in liver Fe, Cu and Co in comparison with the LM + HEP diet, 339.0 vs 150.8, 214.7 vs 68.2, and .24 vs .17 ppm, respectively. Wethers fed the LM + LEP diet had higher ($P < .05$) kidney Fe concentrations compared to the higher energy-protein diet, 641.0 vs 165.2 ppm, respectively. No differences ($P > .05$) were found between diets in the second trial in wool N concentration. High energy-protein diets with low minerals (LM + HEP) had higher ($P < .05$) P and Na concentrations in wool, in comparison with the LM + HEP diet, 171.4 vs 177.2 and 1145.8 vs 795.2.

ppm, respectively. Wool Mg also was higher ($P < .01$) in diet LM + HEP, 68.4 vs 42.8 ppm, respectively. Bone rib Mg concentrations expressed as % ash was higher ($P < .01$) in diet LM + HEP, 0.06 vs 0.89%, respectively. Bone density (g/cc) was higher ($P < .05$) in diet LM + LEP than diet LM + HEP, 1.53 vs 1.39 g/cc, respectively. Differences ($P < .01$) were found between diets LM + LEP and LM + HEP in Ca, P, and Mg retention, 0.72 vs 1.51, 1.32 vs 2.77, 1.12 vs 0.84 g/d, respectively.

Means of Ca retention were 2.74, 1.51, 1.41 and 0.72 g/d for diets HM + HEP, LM + HEP, HM + LEP and LM + LEP, respectively. Means of Mg retention were 2.85, 1.32, 1.12 and 0.84 g/d for diets HM + HEP, HM + LEP, LM + LEP and LM + HEP, respectively. The major route for Ca and P excretion was feces and for Mg excretion, urine. Urinary Mg excretion was higher in wethers fed diets high in minerals. The high energy-protein diets increased Ca, P, and Mg retention for animals receiving either high or low mineral concentrations.

CHAPTER IV
NUTRITIONAL FACTORS AFFECTING MINERAL STATUS AND LONG-TERM CARRY-OVER EFFECTS IN SHEEP. I. MACRO ELEMENTS, ANIMAL PERFORMANCE, HEMOGLOBIN AND HEMATOCRIT

Introduction

Meat and milk production from forage-consuming animals is an important component of tropical agriculture. Approximately half of the world's permanent pastures and half of the cattle population are located in the tropics. However, by standards that have become accepted in temperate climates, tropical livestock productivity is low. Only one-third of the world's meat and one-sixth of its milk products are produced in this region.

Approximately half of the tropics has pronounced wet and dry seasons (ustic soil moisture regime), one-fourth has rainfall distributed throughout the year (udic soil moisture regime), and one-fourth has semiarid or desert climate (aridic soil moisture regime) (Sanchez, 1976). There is little doubt that one of the most seriously limiting factors for beef production in ustic areas is the scarcity of feed during the dry season. Pasture is generally abundant during the rainy season when new shoots or seedlings develop and grow rapidly. The crude protein of some immature indigenous species may reach 8 to 10 percent on a dry matter basis; hence cattle usually gain weight during the rainy season. As physiological maturity approaches, the leaf:stem ratio decreases and consequently the nutritive value declines. Plants become progressively lower in

protein, minerals and soluble carbohydrates and higher in fiber and lignin (Moore and Mott, 1973). These changes decrease the palatability, intake and digestibility of the plants, resulting in decreased energy and protein consumption. In a mature tropical grass, crude protein may fall to a critical level of 3 to 5 percent on a dry matter basis. In Australia, Norman and Stewart (1964) reported that cattle on native pastures needed a supplement of 10.5 g protein/day to prevent weight losses during the dry season.

A deficiency of certain minerals in the forage, particularly P and Ca, may further accentuate the problem. During the dry season the nutritional level of grasses drops below cattle needs for maintenance (energy, protein, P); consequently animals lose weight, conception is delayed, fertility decreases and maturity is prolonged. Cattle gain weight during the rainy season but suffer severe losses during the dry season. This zig zag pattern of weight gains delays slaughtering to an average age of 4 to 10 years with annual live weight gains on the order of 20 to 50 kg/ha with stocking rates of only .05 to .3 animal/ha (Sanchez, 1976). It is generally assumed that P should be fed in conjunction with protein or protein-energy supplements during the dry season. For this reason, P is widely used in practice as an ingredient in winter supplements (Van Niekerk, 1978). Under conditions of the wet-dry tropics, grazing livestock often have abundance of energy-protein supplies, while during the dry season energy-protein sources are inadequate. Effects of energy-protein on mineral supplies is generally unknown; is it possible to supplement for minerals during a short period (i.e., four months) to carry-over

to periods when supplies are inadequate? Different factors influence the productivity of cattle in tropical areas, particularly inadequate nutrition during the long dry period.

The objectives of this study were to investigate the effects of two levels of energy-protein on sheep mineral status and to compare two mineral levels (high and low) on mineral storage and long-term carry-over effects in sheep. In the present experiment animal performance and the nutritional status of macro elements in different animal tissues will be examined.

Experimental Procedure

Forty-eight Rambouillet crosbreed yearling ewe lambs, 8 to 10 months old, average 28.5 kg initial body weight were randomly assigned to four experimental diets in a 2 x 2 factorial arrangement of treatment groups. The duration of the experiment was 18 months (October, 1979 to March, 1981).

The experiment was divided in four periods:

1. Growing period (October, 1979 to March, 1980). Animals were fed for four of this six month period the following semi-purified diets (table 1): 1. low minerals + low energy-protein (LM + LEP); 2. high minerals + low energy-protein (HM + LEP); 3. low minerals + high energy-protein (LM + HEP); and 4. high minerals + high energy-protein (HM + HEP).

High mineral diets were formulated to contain 2 to 30 times the requirements for growing sheep while low mineral diets were approximately the estimated requirements. Levels of energy-protein were .8 x

maintenance for low diets and 1.8 x maintenance for the high diets. Treatments with high minerals were administered for only four months, with these two treatment groups receiving either the respective high or low energy-protein diets for the remainder of the trial.

Ewe lambs were initially weighed, wormed (Levamisole) and allowed to adjust to experimental diets, housed in four pens (12 animals in each pen) representing treatment groups with feed and water available ad libitum.

2. Pre-breeding period (April to July, 1980). Ewes were continued only on the two experimental diets (LM + LEP and LM + HEP). At the end of this period (July 15, 1980) rams were placed in each pen for 45 days for mating purposes.

3. Gestation-parturition period (August, 1980 to January, 1981). Twenty-six ewes were pregnant from a total of 45 exposed ewes. Parturition was from December 9, 1980 to January 24, 1981. Only two sets of twins resulted from ewes of original diet 4 (HM + HEP).

4. Lactation period (January to March, 1981). Ewes and newborn lambs were continued on the two experimental diets low in minerals with two levels of energy-protein (diets 1 and 3, respectively). Lambs were weaned at approximately 60 days of age when the experiment terminated.

During the experiment animals were weighed monthly (17 times) during the 18 month experiment. During the first four months of the trial, lambs on the low energy-protein diets received .85 kg daily and on the high energy-protein diets 1.25 kg per animal daily. In the pre-breeding period, the amounts of feed per animal per day were

adjusted each month according to monthly weight and NRC requirements (Nutrient Requirements of Sheep, 1975). Animals on the low energy-protein treatments received .8 x maintenance requirements while ewes fed the high energy-protein diets received approximately 1.8 x maintenance requirements for energy and protein. In the third period (gestation-parturition) animal requirements were increased to 950 g/d/animal in the low energy-protein diets and 1350 g/d/animal in the high energy-protein diets.

Blood samples were collected in ewes at the end of each experimental period (four times). Blood samples were also collected from both newborn and weaned lambs. Blood serum samples were deproteinized with 10% trichloroacetic acid (TCA) and then analyzed for mineral content according to methods described by Fick et al. (1979). Calcium and Mg were analyzed by atomic absorption spectrophotometry (Perkin Elmer 306) according to procedures recommended by the manufacturers (Anonymous, 1973). Phosphorus was determined by the colorimetric technique described by Harris and Popat (1954). Hemoglobin (Hb) was determined by the modification of the colorimetric method of Martinek (1970) and hematocrit by the microhematocrit method.

Feed samples from experimental diets were taken each two weeks, processed and analyzed for mineral (macro elements) and proximal analysis according to methods described by Fick et al. (1979). Bone biopsies were taken at the beginning of the first period and at the end of each four experimental periods (five times) for Ca, P and Mg analysis. Bone biopsy in sheep was carried out as described by

Little (1972). Also, bone tail samples were taken from both newborn and weaned lambs for mineral analysis. Seven (four female and three male) newborn lambs were sacrificed and metacarpal bone samples were taken for Ca, P and Mg analyses.

Specific gravity was determined for bone samples (rib biopsies, metacarpal and tails) according to the procedure of Little (1972) as modified by Mtimuni (1982). The samples were dried and ether extracted following procedures outlined by Fick et al. (1979) and subsequently analyzed for bone ash, Ca, P and Mg.

Wool samples were collected from ewes only at the beginning of the gestation period and analyzed for N, Ca, P, Mg, Na and K. Nitrogen determination has been described by Technicon Industrial Systems (1978). Milk and colostrum samples were analyzed for Ca, Mg, Na and K by atomic absorption spectrophotometry and P by the colorimetric technique.

As soon as lambs were born, weights were recorded, blood samples were collected from the jugular vein puncture and approximately one inch of tail bone was collected for mineral analyses. Colostrum samples were taken within the first 15 hours after birth and milk samples were collected during middle of the lactation period at an average age of 60 days.

Data were statistically analyzed using a complete randomized design model with a 2 x 2 factorial arrangement of treatments (Snedecor and Cochran, 1973). Data were analyzed by the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS) (Barr et al., 1973) by analysis of variance to test for significance

between levels of energy-protein, levels of minerals and their interaction. Also, pooled analysis was performed to test time effect (period) for different responses.

Results and Discussion

Ewe Lambs

Body weight

Body weight means for ewes fed the four experimental diets are presented in table 14. Appendix table 45 shows means, standard deviation and coefficient of variation of body weights in ewes. Also, appendix table 46 shows analysis of variance-mean squares for body weights. Monthly body weights were measured during the 18 months. Final body weight means were: 42.8, 42.3, 66.8 and 68.6 for ewes fed diets 1, 2, 3 and 4, (LM + LEP, HM + LEP, LM + HEP and HM + HEP), respectively. Ewes fed high energy-protein diets were higher ($P < .001$) in body weights versus ewes fed low energy-protein diets. This difference began at the third monthly weight. No difference ($P > .05$) was found between low and high mineral diets; also, no difference ($P > .05$) was found between interaction levels of energy-protein times levels of minerals in monthly body weights. During this 18-month experiment, levels of energy-protein were more important than levels of minerals for animal performance (monthly body weight).

Hematocrit, hemoglobin and serum macro elements

Means and standard errors of hematocrit, Hb and serum Ca, P, Mg, Na and K in ewes fed four experimental diets during four periods are presented in table 15. Appendix table 47 shows means, standard deviation and coefficient of variation of the same blood parameters. Also,

TABLE 14. MEANS AND STANDARD ERROR OF BODY WEIGHTS IN EWES FED FOUR EXPERIMENTAL DIETS[a]

Weight[b]	DIET 1		DIET 2		DIET 3		DIET 4	
	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error
1	28.07	3.14	29.14	2.43	28.50	1.92	28.39	3.10
2	32.09	2.98	31.32	2.29	33.23	1.87	34.50	2.81
3	32.68	3.27	32.18	2.67	37.61	2.37	38.91	3.76
4	34.23	3.04	33.55	2.60	43.52	2.58	43.82	4.08
5	37.73	2.89	36.70	3.21	46.36	2.39	47.91	4.51
6	38.95	3.61	38.27	2.47	49.23	3.09	51.59	4.55
7	38.64	3.69	37.86	2.75	52.32	3.10	55.18	5.25
8	40.32	3.88	39.55	2.82	54.95	3.60	59.05	5.32
9	40.18	4.36	39.05	3.53	55.35	3.56	60.18	5.95
10	41.68	4.84	39.55	3.66	56.41	3.81	61.41	6.11
11	42.36	4.93	40.05	3.98	58.77	4.33	62.91	5.71
12	46.09	5.42	43.82	4.49	61.69	4.57	66.82	6.11
13	45.45	5.39	42.09	4.37	62.61	5.12	67.68	6.10
14	46.18	4.99	42.07	4.88	65.27	6.23	70.18	5.86
15	42.16	6.34	41.73	5.27	65.25	6.42	68.09	6.17
16	41.59	6.75	40.36	4.79	66.27	6.30	67.68	6.55
17	42.77	7.10	42.27	5.19	66.77	6.56	68.55	6.85

[a] Means based on 12 observations in diet 1 on weights 1st to 17th; 12 observations in diet 2 on weights 1st to 5th, and 11 observations on weights 6th to 17th; 12 observations on diet 3 on weights 1st to 14th, and 11 observations on weights 15th to 17th; 11 observations in diet 4 on weights 1st to 2nd, and 10 observations on weights 3rd to 17th.

[b] Weights measured monthly.

TABLE 15. MEANS AND STANDARD ERRORS OF HEMATOCRIT, HEMOGLOBIN AND SERUM Ca, P, Mg, Na AND K IN EWES FED FOUR EXPERIMENTAL DIETS[a]

Variable	DIET 1		DIET 2		DIET 3		DIET 4	
	Mean	Standard Error (SE)	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error
Period 1								
Hematocrit %	39.50	1.50	42.10	1.43	54.00	1.30	51.60	2.99
Hb %	10.93	0.42	9.81	0.23	11.63	0.30	12.40	0.59
Ca, mg/100 ml	10.23	0.24	11.26	0.23	12.67	0.21	12.76	0.21
P, mg/100 ml	7.96	0.49	5.15	0.37	8.58	0.44	7.58	0.43
Mg, mg/100 ml	2.18	0.07	2.27	0.05	2.54	0.07	2.45	0.09
Na, µg/ml	3467.60	39.37	3150.90	93.07	3086.20	106.85	2911.10	90.87
K, µg/ml	182.25	6.30	157.45	5.49	161.40	7.61	162.80	5.85
Period 2								
Hematocrit %	41.83	0.99	39.18	1.25	44.67	2.66	50.80	1.90
Hb %	14.56	0.79	15.75	0.51	15.27	0.77	17.70	0.42
Ca, mg/100 ml	10.60	0.16	10.83	0.17	9.53	0.42	10.24	0.29
P, mg/100 ml	8.50	0.42	8.28	0.31	9.52	0.49	9.38	0.59
Mg, mg/100 ml	2.33	0.04	2.39	0.05	2.76	0.18	2.32	0.05
Na, µg/ml	3635.00	59.34	3590.10	28.00	3620.25	43.32	3638.70	55.91
K, µg/ml	241.90	5.34	233.00	4.17	228.75	5.44	229.40	5.09
Period 3								
Hematocrit %	37.83	1.95	40.73	1.35	41.75	1.46	43.60	0.98
Hb %	11.88	0.46	12.21	0.51	14.34	0.51	14.20	0.63
Ca, mg/100 ml	12.89	0.41	13.14	0.24	11.24	0.97	12.90	0.33
P, mg/100 ml	9.03	0.50	8.60	0.51	10.40	0.56	9.10	0.75
Mg, mg/100 ml	2.38	0.05	2.60	0.07	3.00	0.31	3.00	0.57
Na, µg/ml	3626.10	44.28	3583.60	106.33	3522.90	110.30	3430.70	84.96
K, µg/ml	191.20	4.77	197.90	4.81	172.30	6.98	179.30	8.38
Period 4								
Hematocrit %	30.50	1.71	30.91	1.77	36.64	1.70	32.10	2.90
Hb %	11.00	0.51	10.86	0.64	13.24	0.46	12.81	0.39
Ca, mg/100 ml	10.11	0.31	10.64	0.28	9.35	0.36	10.14	0.26

TABLE 15 —CONTINUED

Variable	DIET 1 Mean	DIET 1 Standard Error (SE)	DIET 2 Mean	DIET 2 Standard Error	DIET 3 Mean	DIET 3 Standard Error	DIET 4 Mean	DIET 4 Standard Error
P, mg/100 ml	9.33	0.47	9.26	0.49	9.75	0.45	8.90	0.58
Mg, mg/100 ml	2.33	0.05	2.52	0.06	3.05	0.18	2.45	0.06
Na, µg/ml	3233.83	95.16	3408.55	54.05	3259.90	88.32	3372.30	34.50
K, µg/ml	175.25	7.15	180.00	7.94	168.55	6.28	169.40	7.06

[a] Means based on 12 observations in diet 1, periods 1 to 4, except Ca, Na, and K (11), P (10) in period 3; 11 in diet 2, periods 1 to 4, except Hematocrit and Hb (12) in period 1; 12 in diet 3, periods 1 and 2, 7 in period 3, except Hematocrit and Hb (12), 11 in period 4; 10 in diet 4, periods 1 to 4, except Ca, P, Mg, Na, and K (7) in period 3.

analysis of variance-mean squares for the same blood parameters are presented in appendix table 48. In period 1 (growing) significcant ($P < .001$) differences were found in hematocrit, Hb and serum Ca, P, Mg and Na between dietary levels of energy-protein. Blood analyses from ewes fed high energy-protein diets were higher in hematocrit (52.8 vs 40.8%), Hb (12.0 vs 10.4%), Ca (12.7 vs 10.7 mg/100 ml), and P (8.1 vs 6.6 mg/100 ml) versus ewes fed low energy-protein diets. Only Na was higher in serum from ewes fed a low energy-protein diet (3309.2 vs 2998.6 µg/ml) versus ewes fed a high energy-protein diet. Effect of dietary levels of minerals were found only in Ca and Na. Calcium was higher ($P < .05$) in high mineral diets versus low mineral diets (12.0 vs 11.4 mg/100 ml). In contrast, Na was higher in low mineral diets versus high mineral diets (3276.9 vs 3031.0 µg/ml). Interaction between dietary levels of energy-protein x minerals were found only in Hb, Ca, P and K. Hemoglobin and Ca were higher ($P < .05$) in HM + HEP diet (diet 4, P was higher ($P < .05$) in LM + HEP diet (diet 3), and K was higher ($P < .05$) in LM + LEP (diet 1). Means of Hb, Ca, P and K were 10.9, 9.8, 11.6 and 12.4%; 10.2, 11.3, 12.7 and 12.8 mg/100 ml; 7.9, 5.2, 8.6 and 7.6 mg/100 ml and 182.3, 157.3, 161.4 and 162.8 µg/ml for diets 1, 2, 3 and 4, respectively.

In period 2 (breeding) hematocrit was higher ($P < .001$) in ewes fed high energy-protein diets (3 and 4) versus low energy-protein diets (1 and 2) (47.7 vs 40.5%) and Ca was higher ($P < .01$) in low energy-protein diets versus high energy-protein diets (10.7 vs 9.9 mg/100 ml). Serum P was higher ($P < .05$) in low energy-protein diets versus high energy-protein diets (9.0 vs 8.8 mg/100 ml).

Interaction between dietary levels of energy-protein x minerals were found only in hematocrit and serum Mg. Increased levels of energy-protein from low to high in a low and high mineral diet increased significantly ($P < .01$) blood hematocrit (41.8 vs 44.7 and 39.2 vs 50.8%, respectively). Magnesium increased ($P < .05$) only when energy-protein levels were increased in a low mineral diet, but decreased in a high mineral diet (2.3 vs 2.8 and 2.4 vs 2.3 mg/100 ml, respectively). In period 3 (gestation-parturition) hematocrit ($P < .05$), Hb ($P < .001$) and Mg ($P < .05$) were higher in high energy-protein diets versus low energy-protein diets (42.7 vs 39.3%, 14.3 vs 10.0% and 3.0 vs 2.5 mg/100 ml, respectively), while serum K was higher ($P < .01$) in a low energy-protein diet versus high energy-protein diet (194.5 vs 174.8 µg/ml).

In period 4 (lactation) Hb ($P < .001$) and Mg ($P < .01$) were higher in a high energy-protein versus low energy-protein diet (13.0 vs 10.9% and 2.8 vs 2.4 mg/100 ml, respectively), while serum Ca was higher in low energy-protein versus high energy-protein diets (10.4 vs 9.7 mg/100 ml). Calcium was higher ($P < .05$) in a high mineral diet versus low mineral diet (10.4 vs 9.7 mg/100 ml). Interaction between dietary levels of energy-protein and levels of minerals were found only in Mg; in a low mineral diet increased levels of energy-protein increased ($P < .001$) serum Mg from 2.3 to 3.1 mg/100 ml; but in the high mineral diets the mean values were identical (2.5 mg/100 ml) for diets 2 and 4.

Analysis of variance--pooled period comparisons of hematocrit, Hb and serum Ca, P, Mg, Na and K in ewes fed four experimental diets

are presented in appendix table 49. Period effect was different ($P < .01$) for hematocrit, Hb, Ca, P, Mg, Na and in period 1, hematocrit was higher than periods 2, 3 and 4 (46.6, 43.9, 40.9 and 32.5%, respectively). Hematocrit means decreased with increasing animal age. Hemoglobin was higher in period 2 versus period 1, 3 and 4 (14.2, 11.1, 13.1, 11.9%, respectively). Serum Ca was higher in period 3 compared with periods 1, 2 and 4 (12.7, 10.7, 10.3 and 10.1 mg/100 ml, respectively). Phosphorus was higher in period 4, followed by periods 3, 2 and 1 (9.3, 9.2, 8.9 and 7.4 mg/100 ml, respectively). Serum P increased from growing through lactation in ewes fed high minerals with low energy-protein diets (HM + LEP), 5.2, 8.3, 8.6 and 9.3 mg/100 ml, respectively. In ewes fed high minerals with high energy-protein dietary levels, serum P was increased only until breeding period, then decreased in gestation-parturition and lactation periods (7.6, 9.4, 9.1 and 8.9 mg/100 ml, respectively).

Shirley et al. (1968) reported that season or pregnancy and lactation had a significant effect ($P < .01$) on the P content in beef cattle blood. September and December values were generally greater than those obtained in March and June when the cows were advancing in pregnancy. Also, there is no demand for blood P due to lactation in the interval between weaning of calves in September and birth of calves in January to March. Heifers at one year and cows at 14 to 17 years of age had approximately 1 mg/100 ml more ($P < .01$) P in the plasma than intermediate age cows. The authors also reported that Ca in the plasma decreased ($P > .05$) as the cows became older. Values decreased from a range of 11.0 to 11.5 mg/100 ml of plasma during 11 to 17 years of age.

Lane et al. (1968) studied the blood mineral composition in ruminants and found a significant effect of age on P and K ($P < .01$). The young animals (18 to 30 mos) demonstrated the highest mean P level (6.3 mg percent) and the lowest mean K (50 mg percent); in this study age was negatively correlated with P and positively correlated with K. No carry-over effects were observed in hematocrit, Hb and serum minerals (macro elements) in ewes fed the original diets which were high in mineral levels.

Serum Mg increased from period 1 until period 3, then was reduced again in period 4 (2.36, 2.46, 2.68 and 2.59 mg/100 ml, respectively). Serum Na and K increased from period 1 to period 2, then was reduced again in periods 3 and 4 (3165, 3621, 3555 and 3316 g/ml and 166, 233, 187 and 173 g/ml, respectively).

Rib mineral concentrations

Means and standard errors of rib bone mineral concentrations for the four diets within five periods are presented in tables 16, 17, 18 and 19. Appendix table 50 shows the means, standard deviation and coefficient of variation of bone rib mineral concentrations in ewes. Analysis of variance-mean squares for bone rib mineral concentrations in ewes in five periods are presented in appendix table 51.

In period 1 (beginning of growing period) there was a difference ($P < .05$) between dietary levels of energy-protein (low and high) only in Mg expressed as percent of dry, fat-free bone and Mg expressed as percent of ash (0.56 vs 0.47% and 0.90 vs 0.77%, respectively). No difference ($P < .05$) between levels of minerals and no difference in interaction between energy-protein x minerals were found in this period.

TABLE 16. MEANS[a] AND STANDARD ERROR OF BONE RIB MINERAL CONCENTRATIONS IN EWES FED FOUR EXPERIMENTAL DIETS

	DIET 1									
	1		2		3		4		5	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Bone (Dry, Fat-Free)										
Ca, %	21.06	0.42	17.74	0.96	21.35	0.31	20.61	0.55	20.42	0.60
P, %	9.22	1.12	9.51	0.77	11.51	0.57	10.61	0.74	10.61	0.68
Mg, %	0.46	0.01	0.53	0.05	0.67	0.02	0.63	0.03	0.56	0.02
Ash, %	61.40	1.11	53.36	2.90	61.78	0.39	60.70	1.37	60.33	1.40
Ca, % Ash	34.30	0.65	33.29	0.69	34.60	0.53	33.98	0.61	33.80	0.51
P, % Ash	15.02	1.84	17.98	1.28	18.57	0.91	17.50	1.20	17.60	1.05
Mg, % Ash	0.75	0.02	0.99	0.07	1.09	0.03	1.04	0.03	0.92	0.02
Specific gravity, gm/cc	1.84	0.18	1.65	0.009	1.57	0.02	2.18	0.14	1.50	0.04
Ca, mg/cc	387	40.11	296	30.82	336	9.15	447	21.78	307	13.61
P, mg/cc	174	31.83	159	19.63	181	10.54	230	20.14	159	11.51
Mg, mg/cc	8.40	0.79	8.81	1.13	10.24	0.52	13.66	0.78	8.44	0.47
Ca:P ratio	2.42	0.29	1.94	0.18	1.92	0.12	2.02	0.17	2.01	0.14

[a] Means based on 5, 8, 11, 8, and 12 observations on period 1, 2, 3, 4, and 5, respectively on diet 1.

TABLE 17. MEANS[a] AND STANDARD ERROR OF BONE RIB MINERAL CONCENTRATIONS IN EWES FED FOUR EXPERIMENTAL DIETS

	DIET 2									
	1		2		3		4		5	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Bone (Dry, Fat-Free)										
Ca, %	21.15	0.05	21.60	0.27	20.88	0.32	21.03	0.31	22.02	0.32
P, %	11.35	0.15	11.63	0.15	10.63	0.64	11.76	0.13	11.81	0.22
Mg, %	0.49	0.05	0.51	0.01	0.60	0.02	0.58	0.02	0.62	0.02
Ash, %	61.70	0.00	61.48	0.83	59.88	0.64	61.45	0.84	62.07	1.12
Ca, % Ash	34.30	0.10	35.15	0.47	34.91	0.34	34.23	0.27	35.60	0.81
P, % Ash	18.45	0.25	18.91	0.23	17.74	1.09	19.15	0.25	19.05	0.37
Mg, % Ash	0.76	0.08	0.83	0.02	1.00	0.04	0.94	0.04	1.00	0.03
Specific gravity, gm/cc	1.71	0.08	1.77	0.07	1.54	0.04	2.09	0.09	1.58	0.03
Ca, mg/cc	362	18.00	383	16.91	321	11.11	441	21.90	346	5.64
P, mg/cc	195	6.50	206	9.24	164	12.38	246	11.88	186	6.07
Mg, mg/cc	8.25	0.45	9.61	0.94	9.28	0.56	12.14	0.87	9.79	0.42
Ca:P ratio	1.86	0.03	1.86	0.01	2.04	0.18	1.79	0.02	1.88	0.05

[a] Means based on 8 observations in each period on diet 2 except period 1 and 5 with 2 and 5 observations, respectively.

TABLE 18. MEANS[a] AND STANDARD ERROR OF BONE RIB MINERAL CONCENTRATIONS IN EWES FED FOUR EXPERIMENTAL DIETS

	DIET 4									
	1		2		3		4		5	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Bone (Dry, Fat-Free)										
Ca, %	20.65	0.55	19.58	0.65	19.90	0.46	21.30	0.31	21.34	0.66
P, %	11.55	0.35	11.06	0.19	9.51	0.71	11.03	0.68	11.14	1.19
Mg, %	0.60	0.10	0.69	0.03	0.67	0.01	0.67	0.02	0.67	0.01
Ash, %	61.80	1.40	55.90	1.22	58.30	0.97	61.31	0.45	61.52	1.79
Ca, % Ash	33.40	0.10	35.06	0.98	34.14	0.46	34.74	0.51	34.72	0.52
P, % Ash	18.75	0.15	19.85	0.36	16.45	1.39	17.99	1.09	17.94	1.68
Mg, % Ash	0.97	0.14	1.23	0.04	1.15	0.02	1.10	0.04	1.09	0.05
Specific gravity, gm/cc	1.92	0.85	1.33	0.05	1.36	0.06	2.03	0.14	1.42	0.10
Ca, mg/cc	396	27.50	262	16.40	271	17.50	434	33.28	304	28.80
P, mg/cc	222	16.00	148	7.76	128	9.06	228	24.27	161	24.30
Mg, mg/cc	11.55	2.35	9.16	0.60	9.04	0.48	13.80	1.28	9.42	0.69
Ca:P ratio	1.79	0.005	1.78	0.06	2.20	0.22	1.99	0.16	2.02	0.23

[a] Means based on 8 observations in each period on diet 4, except periods 1 and 5 with 2 and 5 observations, respectively.

TABLE 19. MEANS[a] AND STANDARD ERROR OF BONE RIB MINERAL CONCENTRATIONS IN EWES FED FOUR EXPERIMENTAL DIETS

	DIET 3									
	1		2		3		4		5	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Bone (Dry, Fat-Free)										
Ca, %	21.20	0.12	18.37	1.44	17.78	1.96	20.16	0.50	18.94	1.22
P, %	10.77	0.81	10.03	1.25	9.78	1.30	10.93	0.62	10.06	1.57
Mg, %	0.52	0.006	0.73	0.05	0.74	0.09	0.77	0.06	0.64	0.07
Ash, %	62.13	0.32	51.90	4.03	58.99	0.61	59.16	0.88	58.10	3.19
Ca, % Ash	34.13	0.38	35.54	0.80	30.10	3.30	34.10	0.68	32.52	0.75
P, % Ash	17.33	1.41	18.63	1.55	16.58	2.19	18.58	1.27	16.90	2.05
Mg, % Ash	0.84	0.009	1.49	0.19	1.25	0.15	0.29	0.10	1.10	0.10
Specific gravity, gm/cc	1.45	0.08	1.25	0.03	1.41	0.05	1.74	0.10	1.52	0.04
Ca, mg/cc	306	15.93	231	21.13	250	29.78	354	25.20	288	24.20
P, mg/cc	155	10.54	126	16.50	138	19.76	190	14.15	154	26.94
Mg, mg/cc	7.53	0.50	9.19	0.69	10.24	1.40	13.45	1.40	9.70	1.20
Ca:P ratio	1.99	0.15	2.06	0.31	1.91	0.14	1.89	0.13	2.05	0.28

[a] Means based on 3, 7, 8, 8, and 5 observations on period 1, 2, 3, 4, and 5, respectively on diet 3.

In period 2 (end of growing period) specific gravity (1.71 vs 1.29), Mg in ash (.91 vs .36%), Ca expressed as mg/cc (340 vs 183) and P expressed as mg/cc (183 vs 137) were found to be higher ($P < .001$) in bone rib from ewes fed low energy-protein diets versus ewes fed high energy-protein diets. In contrast, Mg in dry, fat-free bone was higher ($P < .001$) in high energy-protein versus low energy-protein diets (.71 vs .52%). In the same period, animals which had received high mineral diets (LEP and HEP) were higher in percent ash ($P < .05$) (58.7 vs 52.6), Ca percent in dry, fat-free bone ($P < .01$) (20.6 vs 18.1), P percent in dry, fat-free bone ($P < .01$) (11.3 vs 9.8), Ca expressed as mg/cc ($P < .05$) (323 vs 264) and P expressed as mg/cc ($P < .05$) (147 vs 143) versus low mineral diets (LEP and HEP), respectively. Only Mg percent in ash was higher ($P < .05$) in low mineral versus high mineral diets (1.24 vs 1.03). No interactions ($P > .05$) were found between dietary levels of energy-protein and levels of minerals in this period.

In period 3 (breeding period) low energy-protein diets were higher in bone specific gravity ($P < .001$) (1.55 vs 1.39), percent ash ($P < .01$) (60.8 vs 58.6), Ca percent of dry, fat-free bone ($P < .05$) (21.1 vs 18.8), Ca mg/cc ($P < .001$) (328 vs 261) and P mg/cc ($P < .01$) (172 vs 133) versus high energy-protein diets. Also, low mineral diets were higher ($P < .05$) in percent ash (60.4 vs 59.1) versus high mineral diets.

In period 4 (gestation-parturition period) bone specific gravity was higher ($P < .05$) in low energy-protein diets versus high energy-protein diets (2.14 vs 1.89). In contrast, Mg percent dry, fat-free bone was higher ($P < .01$) in high energy-protein versus low energy-protein

diets (.72 vs .61); also, Mg percent ash was higher ($P < .001$) in the same diets (1.20 vs .99). Low mineral diets were higher ($P < .05$) in bone Mg concentrations as percent dry, fat-free and percent of ash (.70 vs .63 and 1.16 vs 1.02) versus high mineral diets. No interactions ($P > .05$) between energy-protein x minerals were found in this gestation period.

In period 5 (lactation) only Mg expressed as percent of bone ash was higher ($P < .01$) in high energy-protein versus low energy-protein diets (1.10 vs .96). Calcium, percent dry, fat-free bone was higher ($P < .01$) in high mineral versus low mineral diets (21.7 vs 19.7); also Ca percent of bone ash and Ca, mg/cc were higher ($P < .05$) in high mineral versus low mineral diets (35.2 vs 33.2 and 325 vs 297, respectively). No interactions ($P > .05$) between energy-protein x minerals were found in bone rib mineral concentrations during this lambing period.

Appendix table 52 shows analysis of variance-pooled periods of rib bone mineral concentrations in ewes. There was an effect ($P < .01$) of period in most of the bone parameters. Specific gravity, P percent of dry, fat-free bone and Ca, P, Mg expressed as mg/cc were higher in period 4 (gestation) versus the other four periods (2.01, 11.08, 418.9, 223.9 and 13.3, respectively). Period 1 was higher only in Ca:P ratio (2.12:1). Period 2 was higher in Ca, P and Mg percent of bone ash (34.7, 18.9, 1.12). Period 3 was higher in only Mg percent dry, fat-free bone (.67). Period 5 was higher only in Ca percent dry, fat-free bone (20.8). There was an interaction ($P < .01$) between levels of energy-protein and minerals only in P as a percent of dry, fat-free bone. Significant interaction period x energy-protein

levels were found in Mg percent dry, fat-free bone, percent of Ca and Mg in bone ash ($P < .01$) and Ca mg/cc ($P < .05$). Also, interactions ($P < .01$) in period x minerals were found in Ca, P, Mg dry, fat-free bone, Mg percent ash ($P < .05$) and P expressed as mg/cc ($P < .05$). Interaction period x energy-protein x mineral was found in bone specific gravity ($P < .01$), Mg as percent dry, fat-free bone ($P < .05$), Ca mg/cc ($P < .05$) and Mg mg/cc ($P < .01$).

Vargas (1982) studied the mineral status of cattle in Colombia and found that bone specific gravity for the rainy and dry seasons were as follows: 1.45 and 1.65, with 87 and 55% of the samples, respectively, below the critical level of 1.68 gm/cc for normal cattle (Little, 1972). In a recent Malawi study with cattle, Mtimuni (1982) found 100% of samples on a bone ash basis below critical levels (66.8 for 11th rib ash content in cattle). The author also found that bone Ca concentrations were above the critical level of 24.5% for normal animals (Little, 1972). In contrast, Peducasse (1982) working with cattle in the tropical grasslands in Bolivia, found 100% and 94% of bone samples below critical levels for Ca (24.5%) and P (11.5%), respectively, as expressed on dry, fat-free bone basis. In the present experiment, means for rib bone Ca in ewes fed four experimental diets were below the critical level of 24.5% considered normal for cattle. Means for rib bone P were also below the critical level of 11.5% P for normal cattle (Little, 1982). Most of the studies of mineral status in tropical areas used cattle; it is important to compare sheep bone mineral concentrations between animals from temperate and tropical regions.

In period 1 bone P was higher in ewes fed diets 2 and 4 (high minerals) versus 1 and 3 (low minerals) expressed as percent of dry, fat-free bone, percent of ash and mg/cc (11.35 and 11.55 vs 9.22 and 10.77%; 18.45 and 18.75 vs 15.02 and 17.33%; 195 and 222 vs 174 and 155 mg/cc, respectively). For period 2, bone P continued to be higher in diets 2 and 4 versus diets 1 and 3 (high mineral vs low mineral diets) (11.63 and 11.06 vs 9.51 and 10.03%; 18.91 and 19.85 vs 17.95 and 18.63; 206 and 148 vs 159 and 126 mg/cc, respectively), thus feeding the high minerals resulted in a carry-over for bone P (as percent dry, fat-free bone, percent of ash, mg/cc) in period 2; but for period 3 (gestation-parturition) no carry-over effect of P was observed.

In the case of ash, there would be no carry-over effect comparing diets 2 and 4 (high minerals) versus 1 and 3 (low minerals) because in period 1, diet 3 (LM + HEP) was higher than diet 4 (HM + HEP) (62.1 vs 61.8% of ash, respectively). Bone P provides a direct measure of changes in nutrition of bone. It has been shown (Cohen, 1973) to reflect variation in concentration of P in pasture which varied from .05 to .1% and the bone P varied from 11.2 to 13%. Therefore, bone P has been indicated as the best estimate of P status of grazing ruminants compared to blood and hair P (Cohen, 1973).

Wool, nitrogen and mineral concentrations (macro elements)

Wool samples were taken only during one period (gestation) with means for N, Ca, P, Mg, Na and K concentrations in ewes fed four experimental diets presented in table 20. Means, standard deviation and coefficient of variation of wool N, Ca, P, Mg, Na and K concentrations in ewes are presented in appendix table 53. Analysis of

TABLE 20. MEANS[a] AND STANDARD ERROR OF WOOL NITROGEN, Ca, P, Mg, Na AND K CONCENTRATIONS IN EWES FED FOUR EXPERIMENTAL DIETS

	DIET 1		DIET 2		DIET 3		DIET 4	
Variable	Mean	SE	Mean	SE	Mean	SE	Mean	SE
N, %	13.92	0.13	13.87	0.14	13.97	0.10	13.95	0.18
Ca, µg/g	168.55	13.00	188.55	10.04	204.08	39.61	264.80	76.51
P, µg/g	124.00	11.65	118.00	15.26	125.33	11.28	136.00	10.88
Mg, µg/g	67.09	8.94	68.91	6.95	106.50	19.95	137.20	37.88
Na, µg/g	1101.09	70.47	1185.00	96.98	1364.17	120.05	1468.20	119.43
K, µg/g	3550.00	572.03	3405.09	498.87	4473.17	702.10	3467.70	492.49

[a] Means based on 11, 11, 12 and 10 observations on diets 1, 2, 3 and 4, respectively.

variance-mean squares for wool N, Ca, P, Mg, Na and K concentrations in ewes are presented in appendix table 54. Levels of energy-protein affected only Mg and Na concentrations in wool. Wool Mg and Na concentrations were higher ($P < .05$) in high energy-protein versus low energy-protein diets (121.9 vs 68.0 and 1416 vs 1143 µg/g), respectively. No difference ($P > .05$) due to previous mineral treatments were found. Also, no interactions ($P > .05$) between dietary levels of energy-protein and levels of minerals were found. Kiatoko et al. (1982) studied the nutritional status of beef herds in Florida and reported differences ($P < .05$) in hair P concentrations, which were higher in the dry season than the wet season (219.3 vs 104.8 ppm) and higher in heifers than in mature cows (176.4 vs 160.1 ppm).

Milk and colostrum mineral concentrations (macro elements)

Treatment means for milk and colostrum Ca, P, Mg, Na and K concentrations are presented in table 21. Means, standard deviation and coefficient of variation of milk and colostrum Ca, P, Mg, Na and K concentrations in ewes are presented in appendix table 55. Analyses of variance-mean squares for milk and colostrum Ca, P, Mg, Na and K concentrations in ewes are presented in appendix table 56. No differences ($P > .05$) were found in milk and colostrum Ca, P, Mg, Na and K between high and low energy-protein dietary levels. Differences ($P < .05$) between levels of minerals were found only in milk Mg concentrations. Low mineral diets contain a higher mean Mg concentration than high mineral diets (.128 vs .111 mg/100 ml). No differences ($P > .05$) were found in milk Ca, P, Na and K and colostrum Ca, P, Mg, Na and K concentrations between levels of minerals. Significant

TABLE 21. MEANS[a] AND STANDARD ERROR OF MILK AND COLOSTRUM Ca, P, Mg, Na AND K CONCENTRATION IN EWES FED FOUR EXPERIMENTAL DIETS

	MILK					COLOSTRUM				
Variable	Ca g/100 ml	P g/100 ml	Mg g/100 ml	Na µg/ml	K µg/ml	Ca g/100 ml	P g/100 ml	Mg g/100 ml	Na µg/ml	K µg/ml
Diet 1										
Mean	1.016	0.665	0.134	2745.80	6961.00	0.555	0.670	0.071	1346.57	5130.29
SE	0.158	0.137	0.0078	332.26	784.35	0.056	0.086	0.0044	180.23	710.52
Diet 2										
Mean	1.001	0.812	0.095	2771.50	7191.00	0.565	0.618	0.075	1517.20	4033.40
SE	0.145	0.133	0.0075	376.78	962.22	0.091	0.080	0.007	206.47	498.74
Diet 3										
Mean	1.266	0.959	0.122	3196.25	7569.00	0.635	0.716	0.291	1436.20	5124.80
SE	0.112	0.121	0.004	393.39	818.70	0.175	0.190	0.217	378.67	1639.73
Diet 4										
Mean	1.079	0.818	0.126	3871.00	7460.00	0.404	0.607	0.371	3251.00	3508.00
SE	0.081	0.106	0.009	1034.73	1258.95	0.049	0.073	0.309	1668.05	733.88

[a] Means based on 5 observations on diet 1 and 4 on diets 2, 3 and 4 for milk; and 7 on diet 1, 5 on diet 2 and 3, and 4 on diet 4 for colostrum.

($P < .05$) interactions were found between dietary levels of energy-protein and minerals only in milk Mg concentrations. Increased levels of dietary energy-protein from low to high in a low mineral diet decreased Mg concentrations (.134 vs .122 mg/100 ml), whereas in a high mineral diet Mg concentrations (.095 vs 1.36 mg/100 ml) increased. No interactions ($P > .05$) were found in milk Ca, P, Na and K and colostrum Ca, P, Mg, Na and K concentrations between dietary levels of energy-protein and levels of minerals. Values for Ca, P, Na and K concentrations were numerically higher in milk than colostrum (1.09 vs .55 g/100 ml, .81 vs .67 g/100 ml, 3123 vs 1771 µg/ml and 7276 vs 4559 µg/ml, respectively). Magnesium was higher in colostrum than milk (.18 vs .12 g/100 ml). Akinsoyinu (1981) studied Ca and P in milk and blood serum of the lactating Red Sokoto goat and found that colostrum contained more Ca and P (141.2 and 118.4 mg/100 ml, respectively) than the mature milk (130.4 and 93.2 mg/100 ml).

Newborn and Weaned Lambs

Body weights, hematocrit, hemoglobin and serum macro elements

Means for body weights, hematocrit, Hb and serum Ca, P, Mg, Na and K in lambs from ewes fed four experimental diets is presented in table 22. Means, standard deviation and coefficient of variation of body weights, hematocrit, Hb and serum Ca, P, Mg, Na and K in lambs are presented in appendix table 57. Analyses of variance-mean squares for body weights, hematocrit and Hb in lambs are presented in appendix table 58, and analyses of variance-mean squares for serum Ca, P, Mg, Na and K in lambs are presented in appendix table 59. No sex

TABLE 22. MEANS, STANDARD ERRORS OF BODY WEIGHTS, HEMATOCRIT, HEMOGLOBIN AND SERUM Ca, P, Mg, Na, AND K IN LAMBS FROM EWES FED FOUR EXPERIMENTAL DIETS

Variable	DIET 1			DIET 2			DIET 3			DIET 4		
	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE
Wt.[1] (kg)	9	3.44	0.47	6	3.56	0.48	6	3.26	0.75	7	3.38	0.87
Wt.[2] (kg)	6	15.38	3.05	4	10.11	5.40	2	15.68	4.50	5	11.27	5.54
Hematocrit,[1] %	8	38.81	3.56	6	46.08	3.34	6	48.17	3.57	7	46.86	3.65
Hematocrit,[2] %	6	32.42	2.11	4	31.25	3.06	2	35.00	3.00	5	32.60	1.80
Hemoglobin,[1] %	8	13.14	1.14	6	15.17	0.94	6	14.88	0.90	7	15.84	1.44
Hemoglobin,[2] %	6	11.73	0.90	4	11.20	0.95	2	11.95	0.65	4	10.64	0.75
Ca,[1] mg/100 ml	6	14.15	0.45	5	13.68	0.31	4	15.48	1.64	7	13.76	0.57
Ca,[2] mg/100 ml	6	14.63	2.07	4	11.78	0.77	2	11.50	0.30	5	11.94	0.45
P[1] mg/100 ml	6	8.33	0.79	5	7.78	0.73	4	11.35	2.64	7	7.66	0.56
P[2] mg/100 ml	6	8.67	0.62	4	8.68	0.59	2	9.30	0.10	5	8.26	0.52
Mg,[1] mg/100 ml	6	2.44	0.16	5	2.09	0.13	4	3.14	0.59	7	2.49	0.21
Mg,[2] mg/100 ml	6	3.37	0.61	4	2.44	0.07	2	2.47	0.20	5	2.39	0.08
Na,[1] µg/ml	6	3441.83	57.15	5	3513.80	147.50	4	3416.25	104.84	7	3522.71	35.43
Na,[2] µg/ml	6	3083.33	138.77	4	3008.25	270.22	2	3283.50	16.50	5	3286.80	59.28
K,[1] µg/ml	6	173.00	11.12	5	177.40	7.28	4	184.50	13.30	7	172.57	5.41
K,[2] µg/ml	6	198.17	18.95	4	197.25	10.41	2	194.50	5.50	5	215.40	4.40

[1] Body weight (kg) and sampling at birth.

[2] Body weight (kg) and sampling at weaning (approximately 60 days).

differences ($P > .05$) were found between male and female lambs in body weights (at birth and weaned), hematocrit and Hb. Also, no differences ($P > .05$) were found between levels of dietary energy-protein and minerals. Significant ($P < .01$) interactions were found in sex x energy-protein x minerals in hematocrit and Hb (sampling at birth) and hematocrit ($P < .05$) (sampling at weaning). In newborn lambs, hematocrit and Hb were higher ($P < .01$) in female lambs from ewes fed a high energy-protein diet with high minerals (HM + HEP) and lower in male lambs from ewes fed a low energy-protein diet with low minerals (LM + LEP) (55 vs 32% and 19.2 vs 11.8%, respectively). In weaned lambs, hematocrit was higher ($P < .05$) in male lambs from ewes fed high energy-protein diets with low minerals and lower in male lambs from ewes fed low energy-protein diets with low minerals (38.0 vs 23.0%, respectively). Interactions ($P < .01$) between levels of energy-protein and levels of minerals were found only in serum Ca from newborn lambs. In low and high mineral diets, increased energy-protein levels from low to high increased serum Ca in newborn lambs from 14.4 to 15.5 mg/100 ml and 13.6 to 13.9 mg/100 ml, respectively. In newborn lambs, serum P was higher ($P < .001$) in male lambs from ewes fed high energy-protein diets with low minerals, also serum Mg was higher ($P < .01$) in this group and lower in male lambs from ewes fed low energy-protein diets with low minerals; Mg was lower in male lambs from ewes fed low energy-protein diets with high minerals (15.9 vs 5.2 and 4.2 vs 1.9 mg/100 ml, respectively). In all lambs, hematocrit and Hb concentrations were higher ($P < .05$) in newborn versus the lactation period (44.6 vs 32.5% and 14.7 vs 11.3%, respectively).

Bone tail and metacarpal mineral concentration

Newborn lambs. Means of bone tail mineral concentrations in newborn lambs from ewes fed four experimental diets are presented in table 23. Analyses of variance-mean squares for bone tail mineral concentrations in newborn lambs are presented in appendix table 60. No differences ($P > .05$) were found between sex (male and female lambs) in bone parameters. Also, no differences ($P > .05$) were found between maternal dietary treatments of energy-protein and mineral levels (high and low) in bone tail mineral concentration. Interactions ($P < .05$) between levels of energy-protein and levels of minerals were found only in the Ca:P ratio. Increased levels of energy-protein from low to high in a low mineral diet increased Ca:P ratio (2.57 vs 3.38), in contrast, increased levels of energy-protein from low to high in a high mineral diet decreased Ca:P ratio (2.88 vs 2.21). No interactions ($P > .05$) between sex x energy-protein x minerals were found in bone tail mineral concentrations in newborn lambs. Metacarpal mineral concentrations in newborn lambs from ewes fed four experimental diets are presented in table 24. Only seven newborn lambs were sacrificed to obtain metacarpal bone samples for mineral analyses. Two female lambs from ewes fed low energy-protein diets with high minerals (HM + LEP) were higher in Ca and Mg as expressed as percent of dry, fat-free bone and percent of ash (27.2, .71 and 45.2 and 1.18%, respectively). Specific gravity and percent of ash were higher in six newborn female lambs from ewes fed high energy-protein diets with low minerals (2.24 gm/cc and 66.6% ash). Also, Ca and P expressed in mg/cc were higher in these animals (563 and 298 mg/cc, respectively).

TABLE 23. MEANS AND STANDARD ERROR OF BONE TAIL MINERAL CONCENTRATIONS IN NEWBORN LAMBS FROM EWES FED FOUR EXPERIMENTAL DIETS[a]

Variable	DIET 1		DIET 2		DIET 3		DIET 4	
	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error
Bone Dry Fat Free								
Ca, %	8.833	1.738	11.900	3.491	8.633	1.037	6.500	0.710
P, %	3.750	1.106	5.000	2.198	2.633	0.233	2.940	0.472
Mg, %	0.458	0.166	0.628	0.190	0.403	0.184	0.150	0.473
Ash, %	23.533	2.041	26.675	0.175	23.000	1.769	20.620	0.026
Ca, % Ash	36.683	5.301	44.775	13.290	37.900	5.132	31.420	1.626
P, % Ash	15,383	3.845	18.775	8.297	11.500	0.814	14.240	0.986
Mg, % Ash	1.818	0.600	2.348	0.706	1.837	0.937	0.732	0.148
Specific gravity, gm/cc	1.197	0.0156	1.490	0.188	1.227	0.037	1.206	0.019
Ca, mg/cc	105.667	21.088	168.750	37.750	105.333	9.280	77.800	4.954
P, mg/cc	45.167	13.514	67.750	24.384	32.333	2.028	35.200	3.056
Mg, mg/cc	9.800	2.145	16.525	4.724	4.867	2.122	10.760	3.909
Ca:P ratio	2.617	0.256	2.875	0.471	3.267	0.219	2.200	0.084

[a] Means based on 6, 4, 3, and 5 observations on diet 1, 2, 3, and 4, respectively.

TABLE 24. METACARPAL MINERAL CONCENTRATION IN NEWBORN LAMBS FROM EWES FED FOUR EXPERIMENTAL DIETS

Observation	Diet	Sex[a]	Energy	Mineral	Sp. Gr. gm/cc	Ash %	Ca DFF %	P DFF %	Mg DFF %	Ca % Ash	P % Ash	Mg % Ash	Ca mg/ml	P mg/ml	Mg mg/ml	Ca:P Ratio
1	1	1	1	1	1.78	55.6	19.9	10.2	0.27	35.9	18.4	0.48	355	182	47	1.95
2	2	1	1	2	1.82	60.2	27.2	11.2	0.71	45.2	18.6	1.18	495	204	129	2.43
3	3	1	2	1	1.65	63.1	21.6	13.6	0.30	34.3	21.6	0.47	356	224	49	1.59
4	3	2	2	1	2.07	56.8	20.0	10.7	0.31	35.3	18.8	0.54	415	221	64	1.88
5	3	2	2	1	1.88	59.3	22.3	11.5	0.37	37.7	19.4	0.63	420	216	70	1.95
6	3	1	2	1	2.24	66.6	25.1	13.3	0.45	37.7	20.0	0.67	563	298	100	1.89
7	4	2	2	2	1.85	56.9	21.3	14.1	0.31	37.4	24.7	0.54	394	260	57	1.51

[a] Female, 1; Male, 2.

[1] Low energy, low mineral.

[2] High energy, high mineral.

Weaned lambs. Means, standard deviation and coefficients of variation of bone tail mineral concentrations in newborn and weaned lambs are presented in appendix table 61. Means of bone tail mineral concentrations in weaned lambs from ewes fed four experimental diets are presented in table 25. Analyses of variance-mean squares for bone tail mineral concentrations in weaned lambs are presented in appendix table 62. Differences ($P < .1$) in sex (female and male) were found only in Ca percent dry, fat-free bone (15.8 vs 14.6%, respectively). No interaction ($P > .05$) between sex x energy-minerals, minerals, sex x minerals, energy-protein x minerals and sex x energy-protein x minerals was found in bone tail mineral concentrations in weaned lambs. Bone tail mineral concentration means for weaned lambs were higher than in newborn lambs. Particularly percent of ash, Ca and P as expressed as percent of dry, fat-free bone were higher ($P < .05$) in bone tail from weaned than newborn lambs in diets 1 (LM + LEP), 2 (HM + LEP), 3 (LM + HEP) and 4 (HM + HEP) (46.9, 42,6, 47.2, 45.0% vs 23.5, 26.7, 23.0, 20.6% ash; 15.6, 14.5, 15.9, 15.0% vs 8.8, 11.9, 8.6, 6.5% Ca and 8.4, 8.1, 9.0, 7.9% vs 3.8, 5.0, 2.6, 2.9% P, respectively). In contrast, bone Ca:P ratio was higher ($P < .05$) in tails from newborn lambs than weaned lambs in diets 1, 2, 3 and 4 (2.62, 2.88, 3.27 and 2.20 vs 1.95, 1.83, 1.77 and 1.73, respectively).

Conclusions

Under conditions of the wet-dry tropics, grazing livestock often have an abundance of energy-protein supplies during the wet season, while during the dry season energy-protein sources are inadequate.

TABLE 25. MEANS AND STANDARD ERROR OF BONE TAIL MINERAL CONCENTRATIONS IN WEANING LAMBS FROM EWES FED FOUR EXPERIMENTAL DIETS[a]

	DIET 1		DIET 2		DIET 3		DIET 4	
Variable	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error
Bone Dry Fat Free								
Ca, %	15.583	0.382	14.625	0.477	15.900	0.700	15.000	0.482
P, %	8.400	0.704	8,050	0.409	9.000	0.300	7.933	0.929
Mg, %	0.427	0.015	0.390	0.007	0.440	0.010	0.427	0.020
Ash, %	46,933	0.577	42.625	1.822	47.150	1.150	45.000	1.024
Ca, % Ash	33.183	0.497	34.400	0.892	33.700	0.700	33.250	0.361
P, % Ash	17.933	1.550	18.950	0.897	19.100	0.100	17.450	1.773
Mg, % Ash	0.905	0.023	0.915	0.0477	0.940	0.040	0.947	0.035
Specific gravity, gm/cc	1.173	0.0061	1.268	0.0781	1.195	0.035	1.227	0.012
Ca, mg/cc	182.667	3.997	184.750	8.957	189.000	3.000	183.500	6.520
P, mg/cc	98.500	8.382	101.750	6.981	107.000	-	97.500	11.913
Mg, mg/cc	4.992	0.175	4.900	0.303	5.270	0.270	5.242	0.265
Ca:P ratio	1.953	0.239	1.833	0.050	1.770	0.030	1.747	0.215

[a] Means based on 6, 4, 2, and 6 observations on diet 1, 2, 3, and 4, respectively.

Adequate intake of forages by cattle is essential in meeting mineral requirements. Factors which greatly reduce forage intake, such as low protein (< .7%) content and increased maturity, lignification and stem-to-leaf ratios, all reduce the total minerals consumed. During the wet season, livestock gain weight rapidly since energy and protein supplies are adequate. Associated with rapid growth during the wet season, mineral requirements are high while during the dry season, inadequate protein and energy result in the animal losing weight, thereby greatly limiting mineral requirements (McDowell, 1976). Effect of energy-protein on mineral supplies generally is unknown; it is possible to supplement for minerals during a short period to carry-over to periods when supplies are inadequate.

Mineral status (macro elements) and long-term carry-over effects in sheep were studied in the present experiment. In general, ewes fed high energy-protein diets were higher in body weights than ewes fed low energy-protein diets. No differences were observed in monthly body weights between low and high mineral levels. During this 18-month experiment, levels of energy-protein were more important than levels of minerals for animal performance.

No carry-over effects were observed in hematocrit, Hb and serum minerals (macro elements) in ewes fed for four months on the original experimental diets which are high in mineral levels. Serum P was increased from the growing period throughout the lactation period in ewes fed high minerals with low energy-protein diets (HM + LEP) 5.2, 8.3, 8.6 and 9.3 mg/100 ml, respectively.

In period 1, bone P was higher in ewes fed high mineral versus low mineral diets, expressed as percent dry, fat-free bone, percent of ash and mg/cc. For period 2, bone P continued to be higher in high mineral diets, thus feeding the high minerals resulted in a carry-over effect for bone P in period 2; but for period 3 (gestation-parturition) no carry-over effect of P was observed. Therefore, bone P provides a direct measure of change in nutrition of bone. There was no carry-over effect on bone ash, Ca, Mg expressed as percent of dry, fat-free bone, percent of ash, mg/cc and specific gravity or bone density (gm/cc). Interactions between levels of energy-protein levels from low to high increased serum Ca in newborn lambs from 14.4 to 15.5 mg/100 ml and 13.6 to 13.9 mg/100 ml, respectively. In all lambs, hematocrit and Hb concentrations were higher in newborn versus lactation period (44.6 vs 32.5% and 14.7 vs 11.3%, respectively). Increased levels of energy-protein from low to high in a low mineral diet increased Ca:P ratio from 2.57 to 3.38 in bone tail from newborn lambs; in contrast, increased levels of energy-protein from low to high in a high mineral diet decreased Ca:P ratio from 2.88 to 2.21. Bone tail mineral concentration means for weaned lambs were higher than in newborn lambs, particularly percent of ash, Ca and P as expressed as percent of dry, fat-free bone.

Summary

Effects of two levels of energy-protein on sheep mineral status (macro elements) and two mineral levels (high and low) on mineral storage and long-term carry-over effects in sheep were examined in the present experiment. Forty-eight Rambouillet crossbreed ewe lambs,

8 to 10 months old, averaging 28.5 kg initial body weight were randomly assigned to four experimental diets in a 2 x 2 factorial arrangement of treatment groups.

The duration of the experiment was 18 months (October, 1979 to March, 1981), divided in four periods.

1. Growing period (October, 1979 to March, 1980); animals were fed for four of this six month period the following semi-purified diets: 1. low minerals + low energy-protein (LM + LEP); 2. high minerals + low energy-protein (HM + LEP); 3. low minerals + high energy-protein (LM + HEP); and 4. high minerals + high energy-protein (HM + HEP). High mineral diets were formulated to contain 2 to 30 times the requirements for growing sheep while low mineral diets were close to the estimated requirements. Levels of energy-protein were .8 x maintenance for low diets and 1.8 x maintenance for the high diets. Treatments with high minerals were administered for only four months, with these two treatment groups receiving either the respective high or low energy-protein diet for the remainder of the trial.

2. Breeding period (April to July, 1980); ewes were continued only on the two experimental diets (LM + LEP and LM + HEP) for this four month period.

3. Gestation-parturition period (August, 1980 to January, 1981). Twenty-six ewes were pregnant from a total of 45 exposed ewes. Only two sets of twins resulted.

4. Lactation period (January to March, 1981); ewes and newborn lambs were continued on the two experimental diets low in minerals with two levels of energy-protein.

Blood samples were collected from ewes at the end of each experimental period. Blood samples were also collected from both newborn and weaned lambs and analyzed for hematocrit, Hb and serum minerals (Ca, P, Mg, Na and K). Bone biopsies were taken at the beginning of the first period and at the end of each four experimental periods for Ca, P and Mg analysis. Also, bone tail samples were taken from both newborn and weaned lambs. Seven newborn lambs were sacrificed and metacarpal bone samples were taken for Ca, P and Mg analysis. Wool samples were collected from ewes only at the beginning of the gestation period. Also, milk and colostrum samples were analyzed for Ca, P, Mg, Na and K. Monthly body weights were made during the 18 months. Ewes fed high energy-protein diets were higher ($P < .001$) in body weights versus ewes fed low energy-protein levels. This difference began at the third monthly weight. No difference ($P > .05$) was found between low and high mineral diets. In period 1 (growing), differences ($P < .001$) were found in hematocrit, Hb and serum Ca, P, Mg and Na between dietary levels of energy-protein. Blood analyzed from ewes fed high energy-protein diets were higher in hematocrit (52.8 vs 40.8%), Hb (12.0 vs 10.4%), Ca (12.7 vs 10.7 mg/100 ml), and P (8.1 vs 6.6 mg/100 ml) versus ewes fed low energy-protein diets. Only Na was higher in serum from ewes fed a low energy-protein diet (3309 vs 2999 µg/ml) versus ewes fed a high energy-protein diet. Calcium was higher ($P < .05$) in high mineral diets versus low mineral diets (12.0 vs 11.4 mg/100 ml). In period 2 (breeding), hematocrit was higher ($P < .001$) in ewes fed high energy-protein diets versus low energy-protein (47.7 vs 40.5%). Magnesium

increased ($P < .05$) only when energy-protein levels were increased in low mineral diets, but decreased in a high mineral diet (2.3 vs 2.8 and 2.4 vs 2.3 mg/100 ml, respectively). In period 3 (gestation-parturition), hematocrit ($P < .05$), Hb ($P < .001$) and Mg ($P < .05$) were higher in high energy-protein versus low energy-protein diets (42.7 vs 39.3%, 14.3 vs 10.0% and 3.0 vs 2.5 mg/100 ml, respectively). In period 4 (lactation), Hb ($P < .001$) and Mg ($P < .01$) were higher in a high energy-protein versus low energy-protein diet (13.0 vs 10.9% and 2.8 vs 2.4 mg/100 ml, respectively). Serum P increased from growing through lactation in ewes fed high minerals with low energy-protein diets (HM + LEP) 5.2, 8.3, 8.6 and 9.3 mg/100 ml, respectively. But in ewes fed high minerals with high energy-protein dietary levels, serum P was increased only until the breeding period, then decreased in gestation-parturition and lactation periods (7.6, 9.4, 9.1 and 8.9 mg/100 ml, respectively). No carry-over affects were observed in hematocrit, Hb and serum minerals (macro elements) of ewes fed for four months on the original high mineral diets.

In period 1, bone P was higher in ewes fed diets 2 and 4 (HM + LEP and HM + HEP) versus 1 and 3 (LM + LEP and LM + HEP), expressed as % of dry, fat-free bone, % of ash and mg/cc (11.35 and 11.55 vs 9.22 and 10.77%; 18.45 and 18.75 vs 15.02 and 17.33%; 195 and 222 vs 174 and 155 mg/cc, respectively). For period 2, bone P continued to be higher in diets 2 and 4 versus 1 and 3 (11.63 and 11.06 vs 9.51 and 10.03%; 18.91 and 19.85 vs 17.98 and 18.63%; 206 and 148 vs 159 and 126 mg/cc, respectively), thus feeding the high minerals resulted

in a carry-over effect for bone P in period 2 (breeding); but for period 3 (gestation-parturition) no carry-over effect of P was observed. No differences ($P > .05$) were found in milk and colostrum Ca, P, Mg, Na and K between high and low energy-protein dietary levels. Differences ($P < .05$) between levels of minerals were found only in milk Mg concentrations. Significant ($P < .05$) interactions were found between dietary levels of energy-protein and minerals only in milk Mg concentrations. Increased levels of dietary energy-protein from low to high in a low mineral diet decreased Mg concentrations (.134 vs .122 mg/100 ml) whereas in a high mineral diet Mg concentrations (.095 vs .136 mg/100 ml) increased.

No sex differences ($P > .05$) were found between male and female lambs in body weights (at birth and weaned). In newborn lambs, hematocrit and Hb were higher ($P < .01$) in female lambs from ewes fed a high energy-protein diet with high minerals and lower in male lambs from ewes fed a low energy-protein diet with low minerals (55 vs 32% and 19.2 vs 11.8%, respectively). In low and high mineral diets, increased energy-protein levels from low to high increased serum Ca in newborn lambs from 14.4 to 15.5 mg/100 ml and 13.6 to 13.9 mg/100 ml, respectively. In all lambs, hematocrit and Hb concentrations were higher ($P < .05$) in newborn versus the lactation period (44.6 vs 32.5% and 14.7 vs 11.3%, respectively). In contrast, bone tail mineral concentration means for weaned lambs were higher than in newborn lambs, particularly percent of ash and Ca and P as expressed as percent of dry, fat-free bone (46.9, 42.6, 47.2, 45.0%

vs 23.5, 26.7, 23.0, 20.6% ash; 15.6, 14.6, 15.9, 15.0% vs 8.8, 11.9, 8.6, 6.5% Ca and 8.4, 8.1, 9.0, 7.9% vs 3.8, 5.0, 2.6, 2.9% P, respectively).

CHAPTER V

NUTRITIONAL FACTORS AFFECTING MINERAL STATUS AND LONG-TERM CARRY-OVER EFFECTS IN SHEEP. II. TRACE MINERALS

Introduction

In a pasture-ruminant animal system, a major part of the animal's requirements in terms of energy, protein and vitamins can be satisfied by pasture. Rainfall is the most important climatic parameter for tropical agriculture in terms of both excesses and deficits. During the dry season, grasses mature and become dry, consequently forage quality progressively declines, animals lose weight until at the end of this season may lose as much as 30% of their peak weight in the rainy season (Van Niekerk, 1974).

Fluctuation of nutrient content of the pasture results in the familiar pattern of growth rate of animals on native grasses, that is, rapid growth in the rainy seasons followed by a loss of body weight during the dry season. The deficiencies result in a poor animal performance either directly or through depression of feed intake.

Efforts have been made to increase cattle productivity in these tropical areas. Protein-energy supplementation was reported to alleviate weight loss by cows during the dry season, but supplementation with corn grain or molasses alone or with P as bone meal alone was ineffective (Van Niekerk, 1974). Little (1975) observed that greater improvement of reproductive performance of cows was obtained when supplemental P and protein were provided together.

On the other hand, in many tropical and warm climate regions, mineral deficiencies, imbalances and excesses are severely inhibiting the cattle industry (McDowell, 1976; Conrad and McDowell, 1978). Often livestock grazing in warm climate regions do not receive adequate mineral supplementation and forages rarely satisfy mineral requirements completely.

To have rapid and economical improvement in cattle production in the tropical environment, factors that influence mineral status in ruminants in grazing conditions must be determined. The objectives of this research were to investigate the effect of two levels of energy-protein on sheep mineral status and to compare two mineral levels (high and low) on mineral storage and long-term carry-over effects in sheep. The present study evaluates trace elements.

Experimental Procedure

The experiment was divided in four periods using forty-eight Rambouillet crossbreed ewe lambs, 8 to 10 months old averaging 28.5 kg initial body weight, randomly assigned to four experimental groups in a 2 x 2 factorial arrangement of treatment groups. Experimental periods and diets were described in the previous chapter.

Blood samples were collected in ewes at the end of each experimental period (four times). Blood samples were also collected from both newborn and weaned lambs. Blood serum samples were deproteinized with 10% trichloroacetic acid (TCA) and then analyzed for mineral content according to methods described by Fick et al. (1979). Iron, Cu and Zn were analyzed by atomic absorption spectrophotometry (Perkin Elmer 306) according to procedures recommended by the manufacturers

(Anonymous, 1973). Molybdenum concentrations were analyzed by flameless atomic absorption spectrophotometry using a Perkin Elmer 503 according to procedures recommended by the manufacturer (Anonymous, 1974).

Feed samples from four experimental diets were taken each two weeks, processed and analyzed for trace element contents according to methods described by Fick et al. (1979). Liver biopsies were taken at the beginning of the first period (growing) and at the end of each four experimental periods (five times) for trace elements analyses. The sampling of liver biopsy in sheep was carried out as described by Fick et al. (1979). Samples (.2 to .6 g) were pre-ashed on a hot plate with concentrate nitric acid and then ashed overnight in a muffle furnace at 50° C. Ash was solubilized by digestion first with 50% nitric acid, secondly with 10% nitric acid and finally with distilled water. Solutions were filtered, diluted to the appropriate detection level and analyzed by spectrophotometry for Cu, Fe, Mn and Zn (Perkin Elmer, 1973). Liver Mo and Co were determined with an atomic absorption spectrophotometer equipped with a graphite furnace and D2 corrector. Plasma and liver Se were analyzed by a modification of the fluorimetric method (Whetter and Ullrey, 1978).

Wool samples were collected from ewes only at the beginning of the gestation period. Wool samples were soaked in solution of acationex for 2 d, washed with distilled water, rinsed with 10% hydrochloric acid, washed again with distilled water and dried in an oven at 60° C for 3 d. Milk and colostrum samples (15 and 10 ml, respectively) were dried overnight in an oven at 60° C, then ashed

overnight in a muffle furnace at 500° C. Solutions of wool, milk and colostrum were prepared in the manner described for liver and analyzed for Fe, Cu, Zn, Mn, Mo and Se.

Seven newborn lambs were sacrificed by exsanguination and liver, kidney, heart, muscle and spleen were removed and frozen for subsequent trace elements analyses. Statistical procedures have been described previously in the macro elements experiment.

Results and Discussion

Trace Elements

Blood parameters

Serum Fe, Cu, Zn and Se means for ewes fed four experimental diets during four periods are presented in table 26. Means, standard deviations and coefficient of variation of serum Fe, Cu, Zn and Se in ewes are presented in appendix table 63. Serum Fe increased from period 1 to 4 (1.23, 1.71, 1.87 and 2.25 µg/ml, respectively); in contrast, serum Zn decreased from period 1 to 4 (1.46, 1.41, 1.38 and .92 µg/ml, respectively). No carry-over effects were observed in serum Fe, Cu and Zn in ewes fed for a period of four months the original experimental diets high in mineral levels. Mtimuni (1982) reported that serum Cu was significantly lower in rainy than in dry seasons in all three regions of Malawi (.04, .04, .03 vs .24, .32, .4 ppm). Lebdosaekojo (1977) and Mendes (1977) reported similar results. Analysis of variance-mean squares for serum Fe, Cu, Zn and Se in ewes are presented in appendix table 64.

In period 1 (growing) differences were found between dietary levels of high and low energy-protein in serum Fe ($P < .001$), Cu

TABLE 26. MEANS[a] AND STANDARD ERRORS OF SERUM Fe, Cu, Zn, AND Se IN EWES FED FOUR EXPERIMENTAL DIETS

	Diet 1		Diet 2		Diet 3		Diet 4	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Period 1								
Fe µg/ml	1.05	0.03	1.09	0.03	1.40	0.07	1.91	0.22
Cu µg/ml	0.63	0.04	0.65	0.03	0.50	0.05	0.62	0.06
Zn µg/ml	1.40	0.13	1.26	0.08	1.62	0.03	1.55	0.09
Period 2								
Fe µg/ml	1.66	0.13	1.62	0.16	1.76	0.21	1.79	0.08
Cu µg/ml	0.59	0.05	0.59	0.04	0.36	0.07	0.56	0.10
Zn µg/ml	1.20	0.19	1.18	0.08	1.74	0.16	1.55	0.12
Period 3								
Fe µg/ml	1.81	0.10	1.61	0.08	1.95	0.08	2.27	0.10
Cu µg/ml	0.51	0.04	0.63	0.03	0.45	0.05	0.60	0.08
Zn µg/ml	1.21	0.14	1.26	0.07	1.53	0.12	1.65	0.12
Period 4								
Fe µg/ml	1.91	0.17	1.91	0.26	2.25	0.21	3.06	0.36
Cu µg/ml	0.30	0.04	0.23	0.05	0.36	0.07	0.50	0.11
Zn µg/ml	0.82	0.05	0.87	0.03	1.06	0.05	0.96	0.05
Se µg/ml	0.14	0.02	0.14	0.02	0.16	0.01	0.15	0.02

[a] Means based on 12 observations in diet 1, periods 1, 2, and 4, 11 in period 3; 11 observations in diet 2, periods 1 to 4. Twelve in diet 3, periods 1 and 2, period 3, Fe and Cu (7), Zn (9), period 4, Fe, Cu (11) and Se (10); and 10 observations in diet 4, period 1 and 2, 7 for period 3, and period 4, Fe, Cu (10) and Se (8).

($P < .1$) and Zn ($P < .05$) (1.65 vs 1.07, .56 vs .64 and 1.58 vs 1.33 µg/ml, respectively). Significant interaction ($P < .05$) between levels of energy-protein and minerals were found only in serum Fe in ewes. In low and high mineral diets, increased levels of dietary energy-protein from low to high, increased serum Fe (1.05 to 1.40 and 1.09 to 1.91 µg/ml, respectively).

In period 2 (breeding period), serum Cu was higher ($P < .05$) in ewes fed low energy-protein diets (.59 vs .46 µg/ml) and serum Zn was higher ($P < .01$) in high energy-protein diets (1.64 vs 1.19 µg/ml). In period 3 (gestation-parturition period), serum Fe and Zn were higher ($P < .01$) in a high energy-protein diet versus low energy-protein diets (2.11 vs 1.71 and 1.59 vs 1.24 µg/ml, respectively). Also, from feeding high mineral diets serum Cu concentrations were higher ($P < .01$) than from low mineral diets (.62 vs .48 µg/ml). No interactions ($P > .05$) between dietary levels of energy-protein and minerals were found in this period.

In period 4 (lactation period), treatment differences were found between high and low energy-protein diets in serum Fe, ($P < .01$), Cu ($P < .05$), and Zn ($P < .01$) (1.65 vs 1.91, .43 vs .27 and 1.01 vs .85 µg/ml, respectively). No differences ($P > .05$) were found between previous mineral treatments on levels of minerals in serum Fe, Cu, Zn and Se. Also, no interactions ($P > .05$) between dietary levels of energy-protein and minerals were found in blood parameters in this period. Underwood (1977) gives normal values of plasma Cu in mature normal cattle as .93 µg/ml. Serum Cu levels of .6 ppm were reported to be slightly deficient in cattle (CMN, 1973); also this

committee considered plasma a good indicator of Zn status in animals and suggested above .6 to be a normal level.

Analysis of variance-pooled period of serum Fe, Cu and Zn in ewes fed four experimental diets are presented in appendix table 64. There was a period effect ($P < .01$) in serum Fe, Cu and Zn. Means of serum Cu were .60, .52, .55 and .35 µg/ml for periods 1, 2, 3 and 4, respectively.

Liver trace elements concentrations

Mean liver mineral concentrations for ewes (five periods) fed four experimental diets are presented in tables 27 and 28. Means, standard deviation and coefficient of variation of liver minerals in ewes are presented in appendix table 66. Analyses of variance-mean squares of liver mineral concentrations in ewes are presented in appendix table 67.

In period 1 (growing period) no differences ($P > .05$) were found between dietary levels of energy-protein, levels of minerals and interaction between energy-protein x mineral levels in liver serum Fe, Cu, Zn, Mn, Co and Mo in ewes.

In period 2 (breeding period), liver Fe ($P < .01$) and Co ($P < .05$) concentrations were higher in ewes fed low energy-protein versus high energy-protein diets (382 vs 221 and .43 vs .34 ppm, respectively). Liver Se concentration was higher ($P < .05$) in high energy-protein versus low energy-protein diets (1.98 vs .77 ppm). Liver from ewes fed high mineral diets has higher ($P < .001$) Co and Mo ($P < .01$) concentrations versus low mineral diets (.47 vs .29 and 7.43 vs 3.36 ppm, respectively). Interactions ($P < .001$) between dietary levels

TABLE 27. MEANS[a] AND STANDARD ERROR OF LIVER MINERAL CONCENTRATION IN EWES FED FOUR EXPERIMENTAL DIETS

	Fe ppm	Cu ppm	Zn ppm	Mn ppm	Co ppm	Mo ppm	Se ppm
Diet 1							
Period 1							
Mean	509.00	315.50	279.50	7.70	0.79	2.83	-
SE	378.00	128.50	147.50	0.09	0.36	1.56	-
Period 2							
Mean	321.40	207.20	218.60	7.41	0.33	4.02	1.16
SE	59.52	107.39	92.11	1.09	0.04	0.65	0.28
Period 3							
Mean	396.88	149.66	143.88	8.51	4.54	3.62	-
SE	75.16	52.17	32.29	1.15	1.09	0.40	-
Period 4							
Mean	291.50	162.25	203.50	7.98	0.90	3.14	0.89
SE	56.52	99.90	85.11	1.33	0.25	0.39	0.40
Period 5							
Mean							1.93
SE							0.21
Diet 2							
Period 1							
Mean	311.50	378.00	253.00	9.50	0.57	3.79	-
SE	128.50	220.00	183.00	1.30	0.22	1.08	-
Period 2							
Mean	441.67	63.50	164.33	6.42	0.52	8.40	0.38
SE	71.26	13.32	40.49	0.48	0.05	1.29	0.18
Period 3							
Mean	367.00	49.00	124.33	6.59	4.67	4.57	-
SE	56.98	11.96	41.30	0.60	2.00	0.68	-
Period 4							
Mean	256.00	37.00	138.75	8.42	0.48	3.68	0.86
SE	70.33	5.94	33.44	0.59	0.12	0.49	0.22

TABLE 27—CONTINUED

	Fe ppm	Cu ppm	Zn ppm	Mn ppm	Co ppm	Mo ppm	Se ppm
Period 5							
Mean							1.90
SE							0.07

[a] Means based on 2, 5, 9, 4, and 11 observations on periods 1 to 5, respectively on diet 1, except Se in period 2 and 4 with 2 and 3 observations; and 2, 6, 6, 4, and 10 observations in periods 1 to 5, respectively on diet 2, except Se in period 2 with 3 observations.

TABLE 28. MEAN[a] AND STANDARD ERROR OF LIVER MINERAL CONCENTRATION IN EWES FED FOUR EXPERIMENTAL DIETS

	Fe ppm	Cu ppm	Zn ppm	Mn ppm	Co ppm	Mo ppm	Se ppm
Diet 3							
Period 1							
Mean	169.50	180.00	91.50	7.25	0.45	3.65	
SE	43.50	10.00	19.50	1.12	0.16	1.36	
Period 2							
Mean	198.60	28.20	101.00	7.05	0.24	2.70	1.04
SE	20.68	7.17	6.71	1.07	0.03	0.35	0.20
Period 3							
Mean	249.80	27.40	83.40	7.51	2.26	1.56	
SE	37.62	7.09	7.66	0.94	0.47	0.09	
Period 4							
Mean							1.03
SE							0.02
Period 5							
Mean							1.17
SE							0.54
Diet 4							
Period 1							
Mean	398.33	250.00	298.67	12.07	0.86	4.79	
SE	74.33	72.27	64.54	3.48	0.16	0.49	
Period 2							
Mean	243.80	61.00	96.40	8.79	0.43	6.44	2.93
SE	26.79	12.32	10.11	0.58	0.03	1.41	0.74
Period 3							
Mean	573.00	777.50	165.00	20.04	10.30	9.95	
SE	1.00	692.50	66.00	7.84	5.20	3.85	
Period 4							
Mean	162.00	46.00	92.00	6.01	0.25	1.92	1.24
SE							2.08

TABLE 28—CONTINUED

	Fe ppm	Cu ppm	Zn ppm	Mn ppm	Co ppm	Mo ppm	Se ppm
Period 5							
Mean							1.64
SE							0.47

[a] Means based on 2, 5 and 5 observations in periods 1, 2 and 3 on diet 3, except Se with 2, 2, and 3 observations in periods 2, 4 and 5, respectively; and 3, 5, 2 and 1 observations in periods 1, 2, 3 and 4 on diet 4, except Se with 2, 2 and 3 observations in period 2, 4 and 5, respectively.

of energy-protein and minerals were found only in liver Se concentrations. In a low energy-protein diet, increased mineral levels from low to high decreased liver Se (1.16 to .38 ppm); in contrast, in a high energy-protein diet, increased minerals from low to high increased liver Se concentrations (1.04 to 2.93 ppm).

In period 3 (gestation-parturition period) only liver Mn was higher ($P < .05$) in ewes fed high energy-protein versus low energy-protein diets (13.8 vs 7.6 ppm). Liver Mo concentration was higher ($P < .001$) in ewes fed high levels of minerals versus low minerals (7.26 vs 2.59 ppm). Also, an interaction was found between energy-protein x minerals in liver Fe ($P < .05$), Cu ($P < .01$), Mn ($P < .001$), Co ($P < .05$) and Mo ($P < .001$) concentrations. In a low energy-protein diet, increased mineral levels from low to high decreased liver Fe, Cu and Mn concentrations (397 vs 367; 150 vs 49 and 8.5 vs 6.6 ppm, respectively); in contrast, in a high energy-protein diet, increased mineral levels from low to high increased liver Fe, Cu and Mn concentrations (250 vs 573, 27 to 778 and 7.5 vs 20.0 ppm, respectively). In a low and high energy-protein diet, increased minerals from low to high increased liver Co and Mo concentrations (2.3 vs 10.3 and 1.6 vs 9.9 ppm, respectively) (figure 1).

McDowell et al. (1980) reported the critical level for Fe to be 180 ppm. Cunha et al. (1964) indicated normal liver Fe in cattle varied from 200 to 300 ppm. In the present experiment mean liver Fe concentrations were 253, 309, 371 and 271 ppm for period 1, 2, 3 and 4, respectively. Liver Cu is reported to be the best criterion for assessing Cu status of cattle (CMN, 1973), reflecting dietary

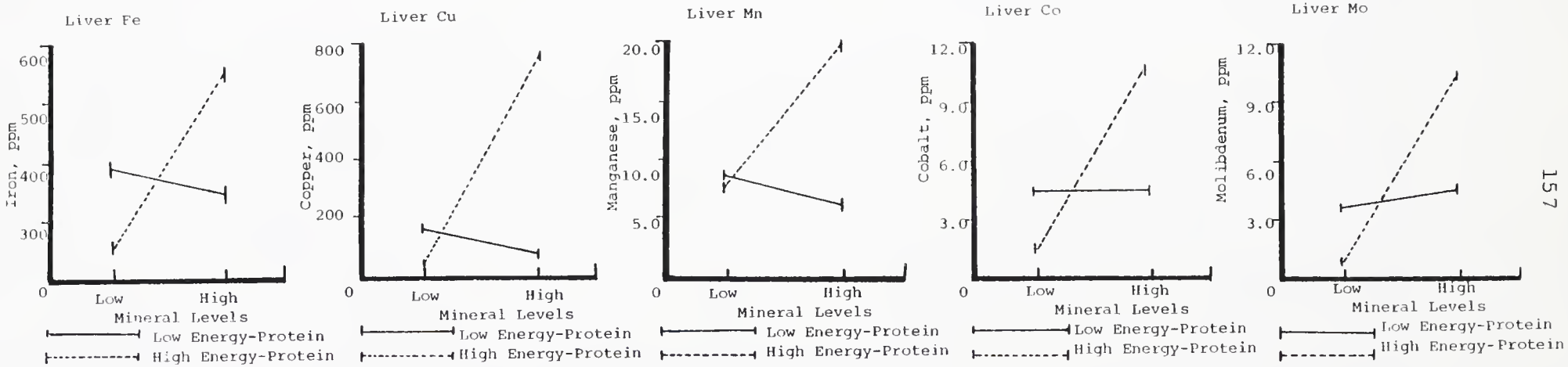

Figure 1. Interactions between levels of energy-protein and minerals in liver Fe, Cu, Mn, Co and Mo concentrations in ewes.

intakes, but may be influenced by dietary concentrations of Mo, Se, Zn, and Ca. Normal concentrations for cattle and sheep are 200--300 ppm, dry basis (Ullrey, 1983). McDowell et al. (1980) reported the critical level for liver Cu to be 25--75 ppm in grazing ruminants. In this experiment mean Cu liver concentrations were 277, 89, 152 and 94 ppm for periods 1, 2, 3 and 4, respectively. Underwood (1962) suggested 125 ppm Zn in the liver as normal for cattle. In this experiment mean liver Zn concentrations were 238, 146, 127 and 162 ppm for periods 1, 2, 3 and 4, respectively. McDowell and Conrad (1977) indicated a critical level of 6--10 ppm Mn in normal liver in cattle. In this experiment mean liver Mn concentrations were 9.5, 7.4, 8.8 and 8.0 ppm for periods 1, 2, 3 and 4, respectively. Vargas (1982) in Colombia reported that Co was the element deficient in largest numbers of liver samples with 50% below the critical level of .05 ppm, particularly in the rainy season in grazing cattle. For the dry season, 44% of liver Co values were below critical level (< .05 ppm); 30% of Cu values (< 75 ppm) and 12% of Zn values (< 84 ppm). During the dry season, 26% of liver Mo values were above 4 ppm. In this experiment mean liver Co concentrations were .69, .39, 4.58 and .64 ppm for periods 1, 2, 3 and 4, respectively. McDowell (1976) reported liver Mo concentrations in excess of 4 ppm, suggestive of dietary excess. In the present experiment, mean liver Mo concentrations were 3.9, 5.5, 4.0 and 3.3 ppm for periods 1, 2, 3 and 4, respectively. Underwood (1979) considers the liver to be a good indicator of Se status, citing a level of .5 ppm Se in liver (fresh basis) in sheep as a marginal one. McDowell

et al. (1978) indicated that a critical level of Se in liver is .25 ppm (dry basis). In this experiment, mean liver Se concentrations were 1.27, .97, and 1.80 ppm for breeding, beginning and end of lambing periods, respectively.

Analysis of variance-pooled periods of liver Fe, Cu, Zn, Mn, Co, and Mo in ewes fed four experimental diets are presented in appendix table 67. Differences ($P < .01$) were found in different periods in liver Mn, Co and Mo concentrations. Manganese was higher in growing period versus breeding, gestation-parturition and lactation periods (9.46, 7.37, 8.81 and 7.95 ppm, respectively), and Mo in breeding period (5.53 ppm) versus growing (3.88), gestation-parturition (3.99) and lactation (3.25) periods. Differences ($P < .05$) were found between dietary levels of energy-protein only in liver Fe concentrations. Differences ($P < .01$) were found also in levels of minerals in liver Mo concentration. Interaction ($P < .01$) between levels of energy-protein x minerals were found in liver Mn, Co and Mo concentrations, also, in Cu ($P < .05$). Interaction ($P < .01$) between period x energy-protein levels were found only in liver Cu concentrations. Interaction ($P < .01$) period x minerals were found in liver Cu and Mo concentrations. Also, interaction ($P < .01$) period x levels of energy-protein x minerals were found in liver Cu.

In the growing period, liver Mo concentrations were higher in ewes fed high mineral (HM + LEP, HM + HEP) versus low mineral (LM + LEP, LM + HEP) diets (3.79, 4.79 vs 2.83, 3.65 ppm, respectively). In the breeding period, liver Mo was increased in ewes fed high mineral diets with low and high energy-protein levels (8.40 and 6.44

ppm, respectively). In the gestation-parturition period, liver Mo concentration was increased from 8.40 to 9.95 ppm only in high mineral with high energy-protein diets and decreased from 6.44 to 4.57 ppm in high minerals with low energy-protein diets. Thus, carry-over effects were observed in liver Mo concentrations of ewes fed high minerals with low energy-protein diets until breeding period and ewes fed high minerals with high energy-protein diets until gestation-parturition period. No carry-over effects were observed in liver Fe, Cu, Zn, Mn, Mo and Se concentrations.

Wool trace elements

Wool Fe, Cu, Zn, Mn, Mo and Se concentrations for ewes are presented in table 29. Means, standard deviation and coefficient of variation of wool Fe, Cu, Zn, Mn, Mo and Se concentrations in ewes are presented in appendix table 69. Wool samples were taken only in one period (gestation-parturition period) with mean Zn, Fe, Cu, Mn, Se and Mo concentrations 948, 13.7, 3.6, .6, .5 and .05 µg/g, respectively. Analysis of variance-mean square for wool Fe, Cu, Zn, Mo and Se concentrations in ewes are presented in appendix table 70. Differences ($P < .01$) were found between dietary levels of energy-protein (high and low) in wool Zn concentrations (1004 vs 892 µg/g, respectively). Interactions ($P < .05$) between levels of energy-protein x minerals were found in Fe. Increased mineral levels from low to high in a low energy-protein diet increased wool Fe concentrations (7.6 vs 22.0 µg/g) but decreased in a high energy-protein diet (14.2 vs 10.7 µg/g). In low mineral diets, increased energy-protein levels increased Fe from 7.6 to 14.2 µg/g, in contrast, high mineral

TABLE 29. MEANS AND STANDARD ERROR OF WOOL Fe, Cu, Zn, Mn, Mo AND Se CONCENTRATIONS IN EWES FED FOUR EXPERIMENTAL DIETS

Variable	DIET 1		DIET 2		DIET 3		DIET 4	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Fe, µg/g	7.55	1.17	22.00	8.06	14.21	2.68	10.75	1.15
Cu, µg/g	3.40	0.21	3.89	0.40	3.36	0.29	3.86	0.38
Zn, µg/g	901.64	58.46	882.55	34.68	995.92	29.20	1012.20	24.86
Mn, µg/g	0.49	0.065	0.643	0.18	0.638	0.086	0.50	0.078
Mo, µg/g	0.036	0.0076	0.057	0.01	0.053	0.010	0.06	0.014
Se, µg/g	0.468	0.186	0.527	0.02	0.514	0.129	0.35	0.04

[a] Means based on 11, 11, 12 and 10 observations on diets 1, 2, 3 and 4, respectively, except Se with 5, 5, 4 and 6 observations, respectively.

diets increased energy-protein levels decreased Fe from 22.2 to 10.7 µg/g.

Levels of some trace elements in hair may be correlated with dietary intake or mineral status of animals (Combs et al., 1982). Combs et al. (1983) reported that Zn content of hair ($P < .01$) increased (204 to 254 µg/g) linearly as levels of dietary Zn increased from 10.3 to 52.9 µg Zn/g diet in experimental rats.

Milk and colostrum

Mean milk and colostrum Fe, Cu, Zn, Mn and Se concentrations as affected by treatment are presented in table 30. Means, standard deviation and coefficient of variation of milk and colostrum Fe, Cu, Zn Mn and Se are presented in appendix table 71. Milk has a higher ($P < .05$) Fe and Mn concentration versus colostrum (4.9 and .76 µg/ml vs 3.2 and .51 µg/ml, respectively). Copper, Zn and Se concentrations were higher ($P < .05$) in colostrum than milk (3.8, 65.4 and .04 vs 3.5, 41.0 and .02 µg/ml, respectively).

Analysis of variance-mean squares for milk and colostrum Fe, Cu, Zn, Mn and Se concentrations in ewes are presented in appendix table 71. Milk Mn concentration was higher ($P < .05$) in high energy-protein versus low energy-protein diets (.91 vs .63 µg/ml). In contrast, Cu concentration in colostrum was higher ($P < .05$) in low energy-protein versus high energy-protein diets (4.47 vs 2.93 µg/ml). No difference ($P > .05$) between levels of minerals and interaction of energy-protein x mineral was found in milk and colostrum trace elements concentration. In cows, sows and goats, Fe supplementation does not appreciably affect milk Fe content (Archibald, 1958). In both rats and sheep, consumption of Cu-deficient diets resulted in

TABLE 30. MEANS[a] AND STANDARD ERROR OF MILK AND COLOSTRUM Fe, Cu, Zn, Mn AND Se CONCENTRATION IN EWES FED FOUR EXPERIMENTAL DIETS

	MILK					COLOSTRUM				
Variable	Fe µg/ml	Cu µg/ml	Zn µg/ml	Mn µg/ml	Se µg/ml	Fe µg/ml	Cu µg/ml	Zn µg/ml	Mn µg/ml	Se µg/ml
Diet 1										
Mean	5.532	3.426	49.20	0.592	0.0177	3.061	4.870	64.714	0.526	0.056
SE	0.782	0.201	26.10	0.099	0.0007	0.355	0.831	9.983	0.081	0.018
Diet 2										
Mean	4.490	2.730	40.250	0.678	0.027	3.324	4.072	82.000	0.392	0.0120
SE	0.507	0.047	13.07	0.084	0.0055	0.621	0.498	12.199	0.062	0.0000
Diet 3										
Mean	4.485	4.058	43.250	0.988	0.0183	2.672	2.372	53.600	0.616	0.020
SE	0.149	0.814	11.294	0.130	0.007	0.512	0.616	15.439	0.152	0.006
Diet 4										
Mean	4.900	3.638	29.250	0.825	0.016	4.075	3.495	61.750	0.515	0.054
SE	0.703	0.735	7.685	0.197	0.004	0.936	0.889	15.787	0.204	0.034

[a] Means based on 5 observations on diet 1, except Se with 2; and 4 observations on diets 2, 3 and 4, except Se with 2 on diet 2 for milk; and 7, 5, 5 and 4 observations for diets 1, 2, 3 and 4, except Se with 4, 1, 3 and 2, respectively for colostrum.

milk with significantly lower than normal Cu concentration (Archibald, 1958). In some dairy animals, Cu supplementation does not appear to increase Cu concentration of milk when the diet is adequate in the element (Archibald, 1958). In dairy animals and in the sow, the Zn content of milk can be increased by dietary supplementation (Miller et al., 1965a). In humans, as well as in cows, the Mn concentration of milk may be elevated by increasing the dietary Mn intake (Archibald, 1958).

Newborn and Weaned Lambs

Blood trace elements

Mean Fe, Cu, Zn and Se concentrations in lambs from ewes fed four experimental diets are presented in table 31. Iron, Cu and Zn were analyzed at birth and at weaning, except Se only at the end of the weaning period. Serum Fe, Cu and Zn decreased from newborn to weaned animals. Means, standard deviation and coefficient of variation of serum Fe, Cu, Zn and Se in lambs are presented in appendix table 73. Serum Fe, Cu and Zn of newborn lambs were higher ($P < .05$) than weaned lambs (3.50 vs 1.75; .20 vs .10; and 1.47 vs .94 µg/ml, respectively).

Analysis of variance-mean squares for serum Fe, Cu, Zn and Se in lambs are presented in appendix table 74. No differences ($P > .05$) were found between sex (male and female) in serum Fe, Cu, Zn and Se concentrations. Interaction sex x levels of energy-protein were found only in serum Zn in weaned lambs. Serum Zn in female and male weaned lambs from ewes fed low mineral levels was higher ($P < .05$) than high mineral levels (1.10 vs 1.08 and 1.20 vs .73 µg/ml,

TABLE 31. MEAN, STANDARD ERRORS OF SERUM Fe, Cu, Zn AND Se IN LAMBS FROM EWES FED FOUR EXPERIMENTAL DIETS

	DIET 1			DIET 2			DIET 3			DIET 4		
	No	Mean	SE	No	Mean	SE	No	Mean	SE	No	Mean	SE
Fe [1] µg/ml	8	3.09	0.50	5	3.08	0.62	4	4.47	1.32	7	3.71	0.73
Fe [2] µg/ml	6	1.79	0.20	4	1.39	0.09	2	2.22	0.65	5	1.80	0.15
Cu [1] µg/ml	8	0.24	0.07	5	0.25	0.08	4	0.14	0.03	7	0.15	0.02
Cu [2] µg/ml	6	0.15	0.05	4	0.12	0.03	2	0.10	0.03	5	0.22	0.09
Zn [1] µg/ml	8	1.61	0.24	5	1.57	0.21	4	1.28	0.28	7	1.36	0.14
Zn [2] µg/ml	6	0.93	0.16	4	0.83	0.21	2	1.10	0.30	5	0.98	0.04
Se [1] µg/ml	4	0.08	0.02	3	0.08	0.04	1	0.06	0.00	5	0.08	0.001

[1] Sampling at birth.

[2] Sampling at weaning (approximately 60 days).

respectively). In female weaned lambs from ewes fed low and high mineral levels, increased energy-protein levels from low to high increased ($P < .01$) serum Fe (1.66 to 2.86 and 1.50 to 1.72 µg/ml, respectively), but in male lambs serum Fe was increased only in low mineral diets (2.43 to 2.57 µg/ml). In female weaned lambs from ewes fed low and high mineral levels, increased energy-protein levels from low to high increased ($P < .001$) serum Zn in low and decreased in a high mineral level (.80 to 1.40 and 1.15 to 1.0 µg/ml). In male lambs from ewes fed low and high mineral levels, increased energy-protein levels from low to high decreased serum Zn in low and increased in high mineral levels (1.60 to .80 and .50 to .97 µg/ml, respectively).

Conclusions

To achieve rapid and economical improvement in cattle production in the tropical environment, factors that influence mineral status in ruminants in grazing conditions must be determined. Protein-energy supplementation was reported to alleviate weight loss by cows during the dry season (Van Niekerk, 1974). Under conditions of the wet-dry tropics, livestock often have an abundance of energy-protein supplies during the wet season while during the dry season, energy-protein sources are inadequate.

Mineral status (trace elements) and long-term carry-over effects of two energy-protein and two mineral dietary levels in sheep were studied in this experiment. No carry-over effects were observed in serum Fe, Cu, Zn and Se in ewes fed the original experimental diets (high minerals) for four months. Concentrations of serum Fe, Zn

and Cu depend mainly on dietary levels of the element, rate of absorption, concentration of other tissue elements, homeostatic control mechanism of the body for the element and the animal species involved (Underwood, 1977).

In the growing period, liver Mo concentrations were higher in ewes fed high mineral versus low mineral diets with two energy-protein levels, low and high (3.79, 4.79 vs 2.83, 3.65 ppm, respectively). In the breeding period, liver Mo was increased in ewes fed high mineral diets with low and high energy-protein levels (8.40 and 6.44 ppm, respectively). In period 3 (gestation-parturition), liver Mo concentration was increased from 8.40 to 9.95 ppm only in animals receiving high minerals with high energy-protein diets, and decreased from 6.44 to 4.57 in those receiving high minerals with low energy-protein diets. Thus, carry-over effects were observed in liver Mo concentrations of ewes fed diets high in minerals with low energy-protein levels until the breeding period and in ewes fed high minerals with high energy-protein levels until the gestation-parturition period. No carry-over effects were observed in liver Fe, Cu, Zn, Mn and Se concentrations.

Mtimuni (1982), working with cattle from Malawi, reported that liver Cu was lower in the rainy season when animal growth requirements increased. Blood Cu levels also increased, which confirmed general observations of mineral concentrations in animal tissues (Lebdosoekojo, 1977; Mendes, 1977; Sousa, 1978). Incidence of Cu deficiency in liver nearly doubled in the rainy season. Vargas (1982) found that mean liver Mo was 11.2 ppm with 50% of the samples above

4 ppm, suggestive of a dietary excess (McDowell, 1976). Ward (1978) indicated that in high Mo diets, liver Cu concentrations may be high but the element may not be biologically available.

Period x energy-protein level interactions were found only in liver Cu concentrations. Period x mineral interactions were found in liver Cu and Mo concentrations. Differences were found between dietary levels of energy-protein (high and low) in wool Zn concentrations (1004 vs 892 µg/g, respectively). Increased mineral levels from low to high in a low energy-protein diet increased wool Fe concentrations from 7.6 to 22.0 µg/g, but decreased in high energy-protein diets from 14.2 to 10.7 µg/g. In low mineral diets, increased energy-protein levels increased Fe from 7.6 to 14.2 µg/g; in contrast, high mineral diets increased energy-protein levels decreased Fe from 22.2 to 10.7 µg/g.

Milk has higher Fe and Mn concentrations than colostrum (4.9 and .76 vs 3.2 and .51 µg/ml, respectively); in contrast Cu, Zn and Se were higher ($P<.05$) in colostrum than in milk (3.8, 65.4 and .04 vs 3.5, 41.0 and .02 µg/ml, respectively). Milk Mn was higher in high energy-protein versus low energy-protein diets (.91 vs .63 µg/ml); in contrast, Cu in colostrum was higher in low energy-protein versus high energy-protein diets (4.47 vs 2.93 µg/ml).

Serum Fe, Cu and Zn decreased from newborn to weaned lambs (3.5, .20, 1.47 to 1.73, .10, .94 µg/ml, respectively).

Summary

Effects of two levels of dietary energy-protein (high and low) on mineral status (trace elements) and two levels of minerals (high

and low) on mineral storage and long-term carry-over effects in sheep were determined in this experiment. Forty-eight Rambouillet crossbreed ewe lambs, 8 to 10 months old, averaging 28.5 kg initial body weight, were randomly assigned to four experimental diets in a 2 x 2 factorial arrangement of treatment groups.

The duration of the experiment was 18 months, divided into four periods: 1) growing period (October, 1979 to March, 1980); 2) breeding period (April to July, 1980); 3) gestation-parturition (August, 1980 to January, 1981); and 4) lactation period (January to March, 1981). Animals were fed the following semi-purified diets for only four of the total six months growing period: (1. LM + LEP; 2. LM + HEP; 3. HM + LEP; and 4. HM + HEP).

In period 1 differences were found between dietary levels of high and low energy-protein in serum Fe ($P < .001$), Cu ($P < .1$) and Zn ($P < .05$) (1.65 vs 1.07, .56 vs .64 and 1.58 vs 1.33 µg/ml, respectively). Significant interactions ($P < .05$) between levels of energy-protein and minerals were found only in serum Fe in ewes. In low and high mineral diets, increased levels of dietary energy-protein from low to high increased serum Fe from 1.05 and 1.40 to 1.09 and 1.91 µg/ml, respectively. In period 2, serum Cu was higher ($P < .05$) in ewes fed low energy-protein diets (.59 vs .46 µg/ml) and serum Zn was higher ($P < .01$) in high energy-protein diets (1.64 vs 1.19 µg/ml). In period 3, serum Fe and Zn were higher ($P < .01$) in a high energy-protein versus low energy-protein diet (2.11 vs 1.71 and 1.59 vs 1.24 µg/ml, respectively). Also, from feeding high mineral diets, serum Cu concentrations were higher ($P < .01$)

than with low mineral diets (.62 vs .48 µg/ml). In period 4, treatment differences were found between high and low energy-protein diets in serum Fe ($P < .01$), Cu ($P < .05$) and Zn ($P < .01$) (1.65 vs 1.91, .43 vs .27 and 1.01 vs .85 µg/ml, respectively). No carry-over effects were observed in serum Fe, Cu and Zn in ewes fed the original high mineral experimental diets for a period of four months. Carry-over effects were observed in liver Mo concentrations of ewes fed high minerals with low energy-protein diets until the breeding period and in ewes fed high minerals with high energy-protein in diets until the gestation-parturition period.

In a low energy-protein diet, increased mineral levels from low to high decreased liver Se from 1.16 to .38 ppm; in contrast, in a high energy-protein diet, increased minerals from low to high increased liver Se concentrations from 1.04 to 2.93 ppm in period 2. In period 3, in a low energy-protein diet, increased mineral levels from low to high decreased liver Fe, Cu and Mn concentrations (397 vs 367, 150 vs 49 and 8.5 vs 6.6 ppm, respectively); in contrast, in a high energy-protein diet, increased mineral levels from low to high increased liver Fe, Cu and Mn concentrations (250 vs 573, 27 to 778 and 7.5 to 20.0 ppm, respectively). In a low and high energy-protein diet, increased minerals from low to high increased liver Co and Mo concentrations (2.3 vs 10.3 and 1.6 vs 9.9 ppm, respectively). Period x mineral interactions ($P < .01$) were found in liver Cu and Mo concentrations. Also, period x levels of energy-protein x mineral interactions ($P < .01$) were found in liver Cu.

Wool samples were taken only in gestation-parturition period with mean Zn, Fe, Cu, Mn, Se and Mo concentrations 948, 13.7, 3.6, .6, .5 and .05 µg/g, respectively. Differences ($P < .01$) were found between dietary levels of energy-protein (high and low) in wool Zn concentrations (1004 vs 892 µg/g, respectively). Interactions ($P < .05$) between levels of energy-protein x minerals were found in Fe. Levels of some trace elements in hair may be correlated with dietary intake or mineral status of animals.

Milk Mn concentration was higher ($P < .05$) in high energy-protein versus low energy-protein diets (.91 vs .63 µg/ml). In contrast, Cu concentration in colostrum was higher ($P < .05$) in low energy-protein versus high energy-protein diets (4.47 vs 2.93 µg/ml). In dairy animals, the Zn content of milk can be increased by dietary supplementation.

Serum Zn in female and male weaned lambs from ewes fed low mineral levels was higher ($P < .05$) than high mineral levels (1.10 vs 1.08 and 1.20 vs .73 µg/ml, respectively). In female weaned lambs from ewes fed low and high mineral levels, increased energy-protein levels from low to high increased ($P < .01$) serum Fe (1.66 to 2.86 and 1.50 to 1.72 µg/ml, respectively), but in male lambs serum Fe was increased only in low mineral diets from 2.43 to 2.53 µg/ml. In female weaned lambs from ewes fed low and high mineral levels, increased energy-protein levels from low to high increased ($P < .001$) serum Zn in low and decreased in a high mineral level (.80 to 1.40 and 1.15 to 1.0 µg/ml). In male lambs from ewes fed low and

high mineral levels, increased energy-protein levels from low to high decreased serum Zn in low and increased in high mineral levels (1.60 to .80 and .50 to .97 µg/ml, respectively).

CHAPTER VI
EFFECT OF VARIABLE ENERGY-PROTEIN AND MINERAL CONCENTRATIONS ON BLOOD METABOLIC PROFILES IN SHEEP

Introduction

Considerable emphasis has been placed by some on the use of livestock metabolic profiles in assessing nutritional status and production potential. Metabolic profiles have been used to diagnose existing disease problems or to monitor health status in a general herd health program. Research to evaluate health status in dairy herds has successfully been based on metabolic blood profiles (Payne et al., 1970; McAdam and O'Dell, 1982). Also, blood parameters in feedlot cattle have been made in attempts to discover and diagnose herd health problems in beef cattle.

Stout et al. (1976) has presented the most comprehensive report on bovine blood profiles. Season, stage of lactation and other factors of variability were considered and guidelines and information applicable to beef cattle were provided. Automated blood analysis has potential use in promoting health and productivity of dairy herds. Four applications of metabolic profiles proposed by Payne and collaborators (1970) are a) monitoring the "metabolic health" of herds, b) helping to diagnose "metabolic problems," c) aiding to diagnose "production disease," and d) selecting individuals that possess "superior metabolism" (Rowlands and Manston, 1976). These workers developed and tested the value of the Compton Metabolic Profile Test (CMPT) in

these applications. The original CMPT involved the analysis of a set of 11 variables in blood samples taken from three groups of seven cows, one near peak lactation, another in midlactation, the other antepartum (dry). Means of variables were calculated for each lactational group in each herd, and this set of means constitutes the metabolic profile (Rowlands and Pocock, 1976).

Many types of automated equipment are available today for accurate, rapid and economical analysis of blood. Auto analyzers are now available to perform multiple blood component assays on a single sample. Examples are the sequential multiple analyzers (SMA) and sequential multiple analyzer with computer (SMAC).

A computer-controlled, high speed sequential analyzer (SMAC) is available in many human diagnostic laboratories for obtaining up to 25 test results from a single sample at a moderate price. According to the scientific literature, the majority of blood components in cattle fall within the ranges found for human blood (Collins, 1978).

Recent studies of Kronfeld et al. (1982) at the University of Pennsylvania reported that multiple regressions on blood variables may be useful in predicting energy and nutrient intakes, and regression analysis may help in selection of blood measurements for inclusion in profiles in dairy herds. The author found that the best serum predictors of ration variables were glutamic-oxaloacetic and glutamic-pyruvic transaminase, cholesterol, P, triglyceride and globulin. Previously studies by Adams et al. (1978) reported that a number of factors limit the usefulness of blood or metabolic profiles. These include sampling problems, low correlations with nutrient intake, inconsistent patterns

in disease, and difficulties in interpretation. The authors reported that differences exist among sites of blood sampling. Serum from blood removed at the mammary vein may be higher in P but lower in Ca and Mg than jugular samples.

English workers (Parker and Blowey, 1976) also have found some significant but low correlations between blood parameters and nutrient intake. Blood profiles are sufficiently sensitive to indicate wide departures from recommended intake in some cases. Adams et al. (1978) reported that blood urea nitrogen was almost 50% less on a ration containing 12.7% vs 17% crude protein on a dry matter basis. Also, blood glucose values appeared to be reduced only 8% when cows were fed forage alone after reaching milk production of 18.2 kg daily. Blood contents have been in reasonably close agreement with estimated intakes from ration evaluation in some problem herds.

This study was designed with three objectives in mind: (1) determine the effect of animal class, age and physiological status (wether lambs, pregnant ewes, and nursing lambs) on metabolic blood profiles; (2) determine the effect of two dietary levels of energy-protein (low = .8 x maintenance; high = 1.8 x maintenance) and two levels of minerals (low and high) on metabolic blood profiles; (3) carry-over effect of high dietary minerals as effected by high and low dietary energy-protein.

Experimental Procedure

Animals sampled in this survery consisted of eleven wether lambs, averaging approximately 50 kg body weight, 45 ewes during three time periods and 14 nursing lambs at the end of the weaning time.

Wether Experiment

Twelve Florida native wether lambs, 12 to 18 months of age, averaging 50.0 kg initial body weight were fed for three months a semi-purified diet (table 1) high in minerals (2 to 30 times maintenance requirements) with two levels of energy-protein (low = .8 x maintenance; high = 1.8 x maintenance). Following the three months, dietary mineral concentrations were reduced and the same animals were fed again for an additional three months a semi-purified diet (table 1), low in minerals with two levels of energy-protein (high and low).

Blood samples were collected at the end of the six month period for metabolic blood profile analysis. Animals were restrained in a squeeze chute and a total of 13 ml of blood were obtained by jugular veni-puncture. Nonheparivized 13 ml SST vacutainer brand evacuated blood collection tubes were used with 18G 1½ gauge needles. After clotting, serum was separated in a centrifuge at 3500 rpm for ten minutes, removed with disposable pipettes to 10 ml tubes and frozen until analyzed. The SMAC 25 unit used in this study was located in Tampa, Florida at Smith Kline Clinical Laboratories (Patterson Coleman Laboratories). Metabolic Profile Tests were glucose, Na, K, Cl, CO_2 content, Balance = Na - (Cl + CO_2), blood urea nitrogen (BUN), creatine, BUN/creatine ratio, uric acid, Ca, P, Fe, total protein, albumin, globulin, A/G ratio, ionized Ca, bilirubin total, alkaline phosphatase, lactic acid dehydrogenase (LDH), serum glutamic-oxalacetic transaminase (SGOT), serum glutamic-pyruvate transaminase (SGPT), total cholesterol and triglycerides.

Ewe Experiment

Forty-eight Rambouillet crossbred ewe lambs, 8 to 10 months of age, averaging 28.5 kg initial body weight were randomly assigned to four experimental groups in a 2 x 2 factorial experiment. Two levels of energy-protein and two levels of minerals were administered to determine the dietary influence on metabolic blood profiles (SMAC 25) parameters. The duration of the experiment was 18 months (October, 1979 to March, 1981) and conducted in an effort to study the nutritional factors affecting mineral status and long-term carry-over effects in ruminants.

The experiment was divided in four periods:

1. Growing period with a duration of six months (October, 1979--March, 1980). Animals were fed for four months of this period the following diets (table 1): a) low minerals + low energy-protein (LM + LEP), Diet #1; b) high minerals + low energy-protein (HM + LEP), Diet #2; c) low minerals + high energy-protein (LM + HEP), Diet # 3; d) high minerals + high energy-protein (HM + HEP), Diet #4. Ewe lambs were housed in four pens (12 animals in each pen) with feed and water available ad libitum.
2. Breeding period (April--July, 1980); ewes were continued on the two experimental diets (LM + LEP and LM + HEP) for four months, housed in two pens. At the end of the four-month period, high mineral diets were eliminated with only diets LM + LEP and LM + HEP being fed.
3. Gestation period (August--December, 1980); ewes continued to be fed only these two experimental diets.

4. Lambing period (January--March, 1981); ewes and newborn lambs were fed the two experimental diets low in minerals with two levels of energy-protein (diets 1 and 3, respectively).

Blood samples were collected three times in ewes and once in lambs; 45 samples from ewes at the end of the first experimental period or growing period (3/9/80); 45 samples from ewes at the end of the third period or gestation period (11/7/80) and 44 samples from ewes at the end of the fourth period or lambing period (3/21/81). Fourteen blood samples were collected from weaning lambs at the end of the lambing period, two months of age (3/2/81). Blood samples from ewes and lambs were collected, prepared and analyzed following the same procedures for metabolic profile analysis which were described previously.

Results and Discussion

Wether Experiment

Effect of energy-protein in blood metabolic profiles (SMAC 25) in wethers are presented in table 32. No differences ($P > .05$) were found between diets 1 and 3 (low in minerals, with low and high energy-protein) in relation to blood glucose, Na, Cl, CO_2 content, Balance = Na - (Cl + CO_2), BUN, creatine, BUN/creatine ratio, uric acid, Ca, P, total protein, ionized Ca, Bilirubin, alkaline phosphatase, LDH, SGOT, SGPT, cholesterol total, and Fe serum. Serum K was higher ($P < .01$) in wethers fed diet 1 (LEP + LM) vs wethers fed diet 3 (HEP + LM), 5.07 vs 4.04 meq/L. Albumin was higher ($P < .05$) in diet 3 vs diet 1, 3.48 vs 3.20 g/DL, respectively. In contrast, globulin was higher ($P < .05$) in diet 1 vs diet 3, 2.91 vs 2.48 g/DL.

TABLE 32. EFFECT OF ENERGY AND PROTEIN IN BLOOD METABOLIC PROFILES (SMAC 25) IN WETHERS[a]

Blood Parameter	DIET 1 (LEP+LM) Mean	SD	DIET 3 (HEP+LM) Mean	SD
Glucose, mg/DL	66.50	2.51	67.20	14.65
Na, meq/L	142.83	5.12	142.40	3.58
Potassium, meq/L	5.07[d]	0.36	4.04[f]	0.35
Chloride, meq/L	104.33	3.61	104.00	2.34
CO_2 Content, meq/L	24.17	2.04	23.00	3.00
Balance = Na - ($Cl+CO_2$) meq/L	14.33	3.93	15.40	3.51
BUN,[g] mg/DL	29.17	32.42	15.40	6.35
Creatine, meg/DL	1.28	0.20	1.26	0.11
BUN/Creatine ratio	23.50	25.34	11.80	4.02
Uric Acid, mg/DL	0.25	0.055	0.28	0.04
Ca, mg/DL	9.80	0.71	8.94	0.63
P, mg/DL	5.45	1.41	6.58	0.62
Total Protein, g/DL	6.12	0.35	5.96	0.23
Albumin, g/DL	3.20[b]	0.18	3.48[c]	0.15
Globulin, g/DL	2.91[b]	0.31	2.48[c]	0.15
A/G[h] Ratio	1.07[d]	0.12	1.34[f]	0.089
Ionized Ca, mg/DL	4.63	0.31	4.34	0.18
Bilirubin, Total, mg/DL	0.10	0.00	0.08	0.045
Alkaline Phosphatase, U/L	131.50	65.61	130.20	15.27
LDH,[i] U/L	412.00	41.03	435.20	29.40
SGOT[j] U/L	62.67	9.91	74.40	10.45
SGPT[k] U/L	12.33	6.83	15.40	8.02
Cholesterol, Total, mg/DL	65.33	5.72	58.20	12.32
Triglycerides, mg/DL	28.33[d]	7.69	56.80[f]	17.37
Fe, Serum, µg/DL	146.83	47.06	142.60	17.66

[a] Means based on six and five observations on diets 1 and 3, respectively.

[b,c] Means in the same row with different superscripts differ ($P < .05$).

[d,f] Means in the same row with different superscripts differ ($P < .01$).

[g] Blood urea nitrogen.

[h] Albumin/globulin ratio.

[i] Lactic dehydrogenase.

[j] Serum glutamic-oxalacetic transaminase.

[k] Serum glutamic-pyruvate transaminase.

Consequently, albumin/globulin ratio was higher ($P < .01$) in diet 3 vs diet 1, 1.34 vs 1.07. Triglycerides were higher ($P < .01$) in blood from wethers fed diet 3 (HEP + LM) vs wethers fed diet 1 (LEP + LM), 45.80 vs 28.33 mg/DL.

Serum glucose levels were variable with a high standard deviation in wethers fed high dietary energy-protein (diet 3). This was expected since blood glucose levels fluctuate with diet, time since food consumption and activity level. Collings (1978) in Florida studied the comparison of bovine blood profiles for Angus, Brahman and Water Buffalo and reported that Brahman showed a higher ($P < .005$) level, 75 mg/dL, of serum glucose than Angus, 65.5 mg/dL. They also reported that yearling animals showed the highest values for Angus, 80.3 mg/dL and Brahman, 91.3 mg/dL. This was probably due to diet because yearling bulls were on concentrate feed containing a higher level of available energy than the grass and hay consumed by the cows. Kaneko and Cornelius (1970a) reported the normal ranges for blood glucose in cows between 35--55 mg/dL and sheep 35--60 mg/dL. Collings (1978) reported averages for three electrolytes, Na, K and CO_2, in Brahmans vs Angus were 144.7 vs 141.9 meq/L; 5.03 vs 4.95 meq/L and 27.5 vs 26.6 meq/L. He also found very little difference for Cl levels between Brahman and Angus cattle (100.1 vs 100.3 meq/L). The parameters of Na, K, CO_2 and Cl reported by Collings (1978) for cattle are in relatively close agreement for wethers in the present experiment. Kaneko and Cornelius (1970b) reported that normal values for serum electrolytes in sheep were Na, 147--156 meq/L; K, 3.9--5.8 meq/L, and Cl, 97--109 meq/L. In this experiment serum

electrolytes of wether lambs fed diet 1 (LEP + LM) and diet 3 (HEP + LM) were in the range previously reported.

The plasma proteins are extremely sensitive to nutritional influence. Vitamins, growth factors, and related substances which affect protein, lipid and carbohydrate metabolism would consequently be expected to influence the plasma protein profile (Kaneko and Cornelius, 1970a). During protein depletion, tissue proteins are utilized to maintain plasma protein (McAdam and O'Dell, 1982). Total serum proteins increased with increase in animal age. These authors also reported that total serum protein concentration was depressed at parturition but rapidly increased to a constant concentration soon after calving in lactating dairy cows. Concentration of total serum protein was greater in mature cows throughout lactation. Peterson and Waldern (1981) studied the effects of feeding regimen (pasture and drylot), physiological stage and age on metabolites in blood serum from Holstein Frisian cows; they found that total protein of serum was higher in dry cows than in either of the two lactating groups, 9.23 vs 7.73 and 7.76 g/100 ml. Collings (1978) reported that Angus cattle were higher ($P < .001$) in serum levels of BUN, 16.2 mg/DL, than Brahman cattle, 10.89 mg/DL. They also reported that all three nitrogenous components, total protein, albumin and BUN were found to show different serum levels between age groups ($P < .001$), total protein increasing with increasing age (5.89 to 7.56 g/DL) and albumin decreasing (4.08 to 3.45 g/DL) with increasing age. The level of serum creatine was higher ($P < .001$) in Brahman cattle, 1.62 mg/dL, than in Angus cattle, 1.36 mg/DL. Calves showing lower

levels than cows, 1.22 vs 1.35 mg/DL for Angus and 1.53 vs 1.71 mg/DL for Brahmans. Intake of water can affect the plasma protein composition. Cattle in a state of thirst possess higher concentrations of albumin and total protein. Two hours after watering the plasma proteins approached normal levels (Kaneko and Cornelius, 1973a). Serum bilirubin levels are only slightly increased in diffuse and severe hepatic disease in the cow. Kaneko and Cornelius (1970a) also found total bilirubin (mg/100 ml) in normal cattle (102 animals) means of .31 ± .17 S.D. These normal values increased to .54 mg/100 ml in severe diffuse hepatic lesions. In the present experiment, serum bilirubin (mg/DL) in wethers fed the two experimental diets were in the normal range (.0--.40).

The serum enzymes measured were alkaline phosphatase (SAP), lactic-dehydrogenase (LDH), serum glutamic-oxalacetic transaminase (SGOT), and serum glutamic-pyruvate transaminase (SGPT). According to Kaneko and Cornelius (1970a) the variation in the concentration of certain serum enzymes as measured by their biochemical activity occur primarily as a result of three processes involving the liver: (1) their elevation due to the escape of enzymes from disrupted hepatic parenchymal cells with necrosis or altered membrane permeability (SGPT, SGOT, LDH); (2) their elevation due to the lack of biliary excretion in obstructive icterus (SAP); and (3) their decrease in concentration in the serum due to impaired synthesis by the liver (choline estearase). Considerable SGOT activity was found in almost all tissues analyzed in mammals, while high SGPT concentrations have been observed only in canine, feline and human hepatic parenchymal cells (Kaneko and

Cornelius, 1970a). Since the livers of mature horses, cattle, sheep and pigs do not contain significant levels of SGPT, only very small elevations in SGPT activity are liver-specific only in small animals as well as all primates studied to date. These authors also reported that SGPT and SGOT expressed as U/L in sheep were 17.4 ± 5.1 and 74.7 ± 13.6, respectively. In the present experiment, values were 62.67 and 74.40 U/L of SGOT in diet 1 (LEP + LM) and diet 3 (HEP + LM), respectively; and 12.33 and 15.40 U/L of SGPT in diets 1 and 3, respectively. Collins (1978) found that season was the only factor affecting ($P < .005$) SGPT levels. Angus cows went from 25.3 U/L in the winter to 33.2 U/L in the summer, while Brahman cows went from 27.9 to 34.0 U/L in the same period. Since enzymes are essential components of many physiological mechanisms, these higher values in summer possibly reflect a higher rate of metabolism in hot weather vs cool weather. In both sheep and cattle, the serum phosphatase activities progressively decreased with age until maturity was reached. A great range of activities (0.3--114.3 King-Armstrong units/100 ml) of Serum Alkaline Phosphatase (SAP) is observed in cattle. The wide range of SAP activities in normal cattle and sheep prohibits its use as an indicator of liver insufficiency or obstructive icterus in these species. For sheep, SAP concentrations range greatly with reported values between 3 and 166 King-Armstrong units/100 ml (Kaneko and Cornelius, 1970a). Serum alkaline phosphatase activity is also elevated in rickets, osteomalacia, osteogenic sarcoma, and secondary hyperparathyroidism. Also, elevation of serum lactic dehydrogenase (LDH) activity have been reported in a variety of hepatic disorders.

Since the serum Fe (SI) determination represents the portion of the transferrin which was bound to Fe^{3+}, the total iron-binding capacity (TIBC) is the sum of the SI and the unbound iron-binding capacity (UIBC) (Kaneko and Cornelius, 1970a). Serum iron (SI) in sheep is between 166--222 µg/DL with the mean and SD of 193 ± 7; and unbound iron-binding capacity (UIBC) of 141 ± 10. In the present wether experiments, lower serum Fe levels were found at 146.8 and 142.6 µg/DL in diets 1 and 3, respectively.

There are large differences in the blood lipid components among species, among individuals within species and at various times within an individual. Factors influencing those lipid levels (cholesterol, triglycerides) include the quantity and type of lipid in the diet, time after consumption of food, state of health of the animal, age, hormone balance or imbalance, energy needs and others (Swenson, 1970). Particular levels of triglycerides can vary greatly, depending in large part on dietary intake, storage in or mobilization from adipose tissues and on synthesis by the liver. No differences ($P > .05$) were found in total cholesterol, mg/DL in serum from wethers fed diets 1 and 3 (LEP + LM and HEP + LM), but animals receiving the high energy-protein diet had higher ($P < .01$) triglyceride levels, 56.8 vs 28.3 mg/DL. Appendix table 75 shows individual values.

Ewe Experiment

Means for metabolic blood profiles (SMAC 25) in ewes fed four experimental diets are presented in tables 33--34, while data for ewes fed diets during three periods are presented in table 35. Analysis of variance and mean squares for blood metabolic profiles are presented

TABLE 33. MEANS[a] AND STANDARD ERRORS OF METABOLIC BLOOD PROFILE (SMAC 25) IN EWES FED FOUR EXPERIMENTAL DIETS

	DIET 1						DIET 2					
	PERIOD 1		PERIOD 2		PERIOD 3		PERIOD 1		PERIOD 2		PERIOD 3	
	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error
Glucose, mg/DL	69.00	3.28	57.83	1.22	59.92	2.94	60.27	1.83	59.63	2.87	64.18	6.52
Na, meq/L	143.67	1.10	144.42	0.58	149.50	0.44	141.27	1.05	144.55	0.59	148.73	0.49
K, meq/L	5.18	0.32	5.49	0.08	5.21	0.11	5.08	0.19	5.25	0.18	5.13	0.14
Chloride, meq/L	106.75	0.68	105.92	0.58	108.67	0.72	105.09	0.74	104.27	0.57	108.91	1.32
CO_2 Content, meq/L	24.42	0.58	28.83	0.46	25.75	0.83	24.73	0.54	24.55	0.47	23.18	1.44
Balance = Na-(Cl+CO_2) meq/L	12.00	0.90	14.67	0.78	15.08	1.21	11.45	0.97	15.73	0.69	16.64	1.78
BUN, mg/DL	11.83	0.99	9.33	0.92	14.00	0.92	10.18	0.90	7.55	0.43	12.73	0.65
Creatine, mg/DL	1.03	0.03	1.28	0.05	1.20	0.08	0.89	0.03	1.40	0.08	1.33	0.08
BUN/Creatine ratio	11.50	0.99	7.58	0.96	12.58	1.44	11.55	0.99	5.55	0.41	10.00	0.88
Uric Acid, mg/DL	0.30	0.04	0.27	0.04	0.20	0.00	0.23	0.04	0.29	0.03	0.20	0.00
Ca, mg/DL	8.70	0.15	9.15	0.19	8.22	0.23	10.05	0.14	9.21	0.18	8.48	0.20
P, mg/DL	7.37	0.28	7.67	0.34	10.11	0.81	4.59	0.20	7.88	0.46	9.72	0.73
Total Protein, g/DL	6.53	0.10	6.44	0.13	6.20	0.19	6.41	0.15	6.38	0.14	6.21	0.15
Albumin, g/DL	3.23	0.08	3.31	0.08	3.02	0.13	3.11	0.10	3.30	0.09	3.10	0.16
Globulin, g/DL	3.29	0.09	3.13	0.10	3.60	0.46	3.30	0.11	3.08	0.07	3.13	0.07
A/G ratio	0.98	0.04	1.02	0.04	0.91	0.04	0.91	0.05	1.03	0.02	0.96	0.07
Ionized Ca, mg/DL	3.94	0.06	4.20	0.08	3.83	0.10	4.64	0.02	4.24	0.08	3.95	0.11
Bilirubin, Total, mg/DL	0.11	0.008	0.14	0.02	0.09	0.008	0.10	0.01	0.11	0.01	0.10	0.00
Alkaline Phosphatase, U/L	214.67	24.32	180.17	18.85	160.83	21.95	202.10	17.89	202.18	23.91	220.82	31.18
LDH, U/L	514.00	22.55	389.50	28.48	274.25	15.26	524.91	14.72	471.55	53.86	552.27	201.07
SGOT, U/L	97.92	5.50	70.17	7.45	75.83	6.21	80.10	3.49	92.18	26.58	221.55	101.22
SGPT, U/L	17.92	3.16	21.25	3.11	17.67	4.93	21.27	3.73	25.00	4.12	25.73	8.64
Cholesterol Total, mg/DL	122.17	42.46	86.33	3.71	83.08	4.56	88.36	3.72	85.91	3.71	81.10	5.33
Triglycerides, mg/DL	20.17	3.94	43.08	5.31	21.25	3.17	12.54	0.95	38.10	7.12	27.73	5.17
Fe, Serum, mg/DL	180.75	10.07	224.08	15.17	147.08	13.00	174.91	8.00	212.73	10.60	174.36	16.27

[a] Means based on 12 observations on diet 1 and 11 on diet 2.

TABLE 34. MEANS[a], STANDARD ERRORS OF METABOLIC BLOOD PROFILE (SMAC 25) IN EWES FED FOUR EXPERIMENTAL DIETS

	DIET 3						DIET 4					
	PERIOD 1		PERIOD 2		PERIOD 3		PERIOD 1		PERIOD 2		PERIOD 3	
SMAC 25	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error
Glucose, mg/DL	76.42	3.64	74.67	4.06	69.33	3.14	69.10	5.67	78.50	2.20	80.89	10.43
Na, meq/L	144.08	0.99	146.42	0.84	150.07	1.02	142.30	0.96	145.00	0.71	149.00	0.62
K, meq/L	5.03	0.14	5.29	0.21	5.07	0.19	4.69	0.10	5.02	0.10	4.96	0.17
Chloride, meq/L	103.75	0.60	106.08	0.94	110.58	0.80	103.30	0.99	105.80	0.89	109.67	0.80
CO_2 Content, meq/L	22.17	0.56	19.25	0.77	12.17	1.12	21.80	0.63	18.60	1.30	19.56	1.03
Balance = Na-(Cl+CO_2) meq/L	18.17	0.87	21.08	0.82	22.17	1.46	17.20	0.87	20.60	1.28	19.78	1.49
BUN, mg/DL	24.17	0.96	17.17	1.21	23.00	1.78	26.00	1.55	18.00	0.77	21.11	2.53
Creatine, mg/CL	1.03	0.05	1.47	0.06	1.32	0.07	1.06	0.05	1.40	0.06	1.37	0.04
BUN/Creatine ratio	23.83	1.32	13.33	1.27	17.00	1.48	24.70	1.33	13.10	0.75	15.22	1.50
Uric Acid, mg/DL	0.21	0.03	0.38	0.03	0.20	0.00	0.17	0.02	0.30	0.04	0.21	0.01
Ca, mg/DL	7.98	0.30	7.92	0.36	7.77	0.41	8.32	0.15	8.19	0.29	8.03	0.39
P, mg/DL	9.33	0.49	8.88	0.76	11.61	0.84	8.55	0.20	8.47	0.35	9.74	1.09
Total Protein, g/DL	6.65	0.13	7.25	0.11	7.25	0.12	6.32	0.12	7.10	0.11	7.12	0.14
Albumin, g/DL	3.59	0.04	3.86	0.06	3.85	0.09	3.44	0.06	3.83	0.06	3.83	0.10
Globulin, g/DL	3.06	0.11	3.40	0.09	3.42	0.11	2.88	0.08	3.27	0.09	3.29	0.11
A/G ratio	1.13	0.04	1.26	0.19	1.11	0.05	1.16	0.01	1.12	0.03	1.12	0.05
Ionized Ca, mg/DL	3.58	0.16	3.34	0.17	3.28	0.19	3.82	0.07	3.52	0.12	3.43	0.18
Bilirubin, Total, mg/DL	0.15	0.02	0.13	0.01	0.11	0.008	0.12	0.02	0.10	0.01	0.10	0.00
Alkaline Phosphatase, U/L	234.25	19.85	207.83	31.88	166.25	20.00	207.40	17.21	174.90	19.60	158.33	22.00
LDH/ U/L	635.83	31.45	581.25	53.53	356.67	27.25	584.30	16.44	531.30	26.10	328.89	22.46
SGOT, U/L	130.17	8.72	92.92	6.66	95.92	6.42	108.10	8.27	87.20	4.63	91.56	5.79
SGPT, U/L	22.25	3.79	20.33	2.16	13.67	1.94	24.60	6.38	24.70	2.29	21.89	6.50
Cholesterol Total, mg/DL	78.50	4.77	80.67	3.21	77.67	3.79	76.60	6.55	86.30	4.11	76.33	6.71
Triglycerides, mg/DL	12.33	1.28	16.75	2.97	20.83	3.17	17.20	1.63	15.00	3.44	19.89	5.68
Fe, Serum, mg/DL	198.92	11.93	192.17	14.93	180.58	18.21	255.10	11.44	222.70	10.55	203.56	14.44

[a] Means based on 12 observations on diet 3, and 20 on diet 4.

TABLE 35. MEANS,[a] STANDARD DEVIATIONS AND COEFFICIENT OF VARIATION OF METABOLIC BLOOD PROFILE (SMAC 25) IN EWES FED FOUR EXPERIMENTAL DIETS BY PERIODS

SMAC 25[1]	FIRST PERIOD			SECOND PERIOD			THIRD PERIOD		
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
Glucose, mg/DL	68.87	12.52	18.18	66.87	9.52	14.23	67.84	19.34	28.50
Na, meq/L	142.89	3.48	2.43	145.11	2.33	1.61	149.43	2.33	1.56
K, meq/L	5.01	0.73	14.49	5.28	0.52	9.91	5.10	0.52	10.16
Chloride, meq/L	104.78	2.52	2.40	105.53	2.56	2.43	109.45	3.12	2.85
CO_2 Content, meq/L	23.31	1.94	8.32	21.62	2.63	12.16	21.50	3.75	17.43
Balance = Na-Cl+CO_2) meq/L	14.67	3.04	20.70	17.96	3.00	16.73	18.36	4.96	26.99
BUN, mg/DL	17.87	3.74	20.96	13.44	3.05	22.68	17.59	5.09	28.95
Creatine, mg/DL	1.00	0.13	12.87	1.39	0.21	15.29	1.30	0.24	18.56
BUN/Creatine ratio	17.73	3.91	22.03	9.84	3.14	31.86	13.68	4.50	32.90
Uric Acid, mg/DL	0.23	0.105	45.87	0.31	0.11	34.97	0.20	0.015	7.37
Ca, mg/DL	8.75	0.69	7.83	8.62	0.90	10.41	8.12	1.05	12.95
P, mg/DL	7.48	1.00	14.76	8.22	1.75	21.23	10.35	2.84	27.50
Total Protein, g/DL	6.48	0.42	6.48	6.79	0.41	6.10	6.68	0.51	7.57
Albumin, g/DL	3.34	0.25	7.43	3.57	0.23	6.52	3.43	0.41	12.04
Globulin, g/DL	3.14	0.34	10.89	3.22	0.29	9.15	3.37	0.88	26.26
A/G ratio	1.04	0.14	13.74	1.11	0.35	31.69	1.02	0.18	17.25
Ionized Ca, mg/DL	3.99	0.33	0.61	3.83	0.41	10.71	3.63	0.49	13.47
Bilirubin, Total, mg/DL	0.12	0.06	51.27	0.12	0.05	41.39	0.10	0.02	21.41
Alkaline Phosphatase, U/L	215.20	68.41	31.79	191.76	82.41	42.98	176.80	80.33	45.44
LDH, U/L	564.78	77.44	13.71	492.20	145.32	29.52	377.41	339.58	89.98
SGOT, U/L	104.42	22.91	21.94	85.40	47.58	55.71	120.95	168.82	139.57
SGPT, U/L	21.38	14.32	66.97	22.69	10.16	44.76	19.45	19.34	99.42
Cholesterol Total, mg/DL	92.13	77.53	84.15	84.71	12.30	14.52	79.73	16.58	20.80
Triglycerides, mg/DL	15.56	7.97	51.22	28.60	16.77	58.65	22.48	14.07	62.58
Fe, Serum, mg/DL	200.69	35.26	17.57	213.82	44.77	20.94	174.59	52.49	30.06

[1] See Table for metabolic blood profile identification.

[a] Means based on 45 observations on Period 1 and 2, and 44 observations on Period 3.

in appendix table 76. In the first period (end of growing period), ewes fed high energy-protein diets (3 and 4) HEP + LM and HEP + HM, respectively, were found to be higher ($P < .05$) in serum glucose (72.8 vs 64.6 mg/DL), balance = Na - (Cl + CO_2) (17.7 vs 11.7 meq/L), BUN (25.1 vs 11.0 mg/DL), creatine (1.04 vs 0.96 mg/DL), BUN/creatine (24.2 vs 11.5), P (8.9 vs 6.0 mg/DL), albumin (3.52 vs 3.17 g/DL), A/G ratio (1.15 vs 0.93), LDH (610 vs 519 U/L), SGOT (119 vs 89 U/L) and Fe (227.0 vs 177.8 µg/DL) than ewes fed low energy-protein diets (1 and 2) LEP + LM and LEP + HM, respectively. Serum CO_2 (24.6 vs 22.0 meq/L), Cl (105.9 vs 103.5 meq/L), uric acid (0.26 vs 0.19 mg/DL), Ca (9.4 vs 8.1 mg/DL), globulin (3.30 vs 2.97 g/DL) and ionized Ca (4.30 vs 3.70 mg/DL) were found to be higher ($P < .05$) in ewes fed low energy-protein diets (1 and 2) vs ewes fed high energy-protein diets (3 and 4). Serum from ewes fed high mineral diets (2 and 4) LEP + HM, HEP + HM were found to be higher ($P < .05$) in Ca (9.2 vs 8.3 mg/DL), ionized Ca (4.23 vs 3.76 mg/DL) and Fe (215.0 vs 189.8 µg/DL) than ewes fed low mineral diets (1 and 3) LEP + LM, HEP + LM, respectively.

For animals fed low mineral diets, higher ($P < .05$) concentrations were found for glucose (72.7 vs 64.7 mg/DL), Na (143.9 vs 141.8 meq/L) and P (8.4 vs 6.6 mg/DL) than ewes fed high mineral diets. Also there was an interaction ($P < .05$) between levels of energy-protein and levels of minerals in serum creatine, Ca, ionized Ca and triglyceride; also there was an interaction ($P < .01$) between levels of energy-protein and levels of minerals in serum P and Fe. Figure 2 shows that serum creatine levels were 1.03, 0.89, 1.03 and

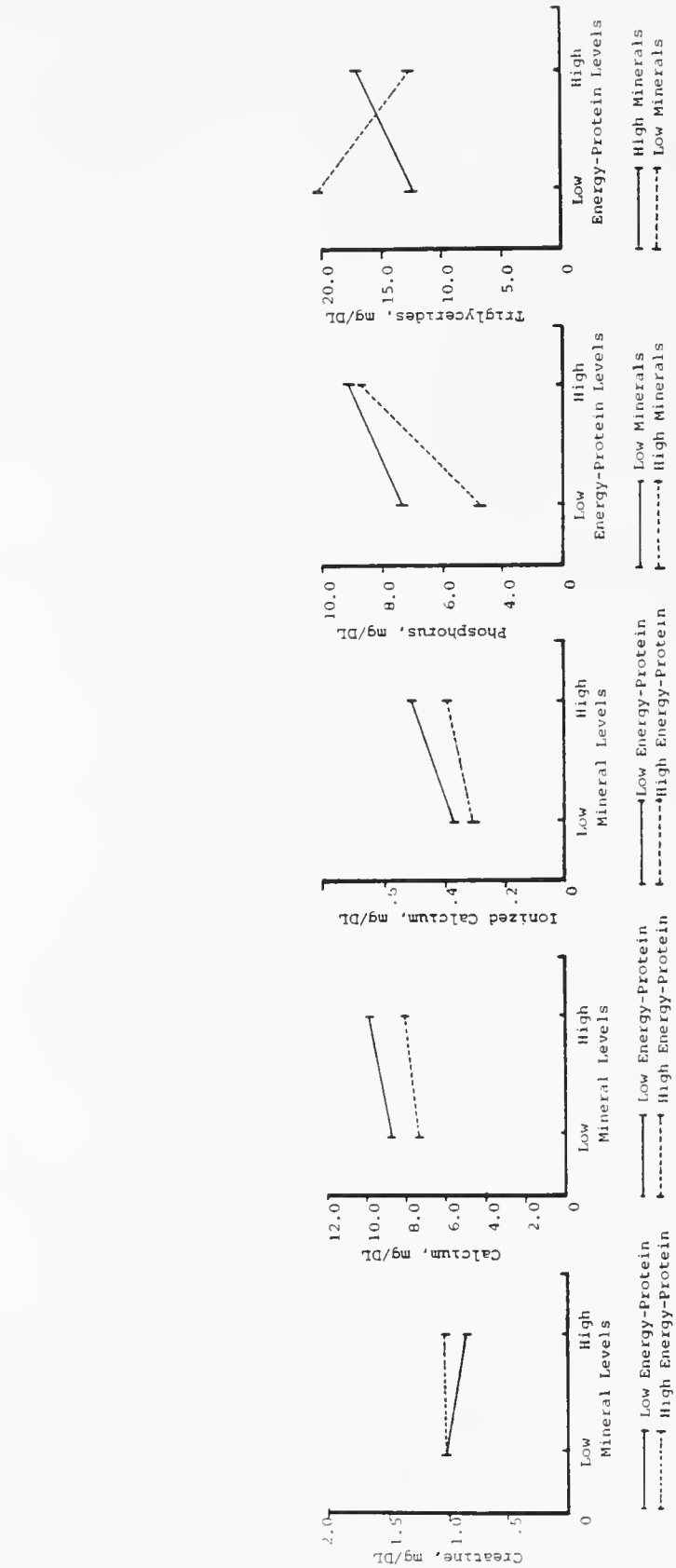

Figure 2. Interactions between levels of energy-protein and levels of minerals in serum creatine, Ca, ionized Ca, P and triglycerides (SMAC 25) in ewes fed four experimental diets (period 1).

1.06 mg/DL for diets 1, 2, 3 and 4, respectively; that means if increased levels of energy-protein in low mineral diets, there was no change in serum creatine values; but if increased levels of energy-protein in high mineral diets, resulted in a significant increase in serum creatine--1.03 to 1.06 mg/DL. Serum Ca was higher ($P < .05$) when levels of minerals were increased in low and high energy-protein diets: 8.7, 10.1, 7.8 and 8.3 mg/DL for diets 1, 2, 3 and 4, respectively. For serum P, levels of energy-protein were more important than levels of minerals. Compared to the low mineral diet increased levels of energy-protein increased serum P (7.4 vs 9.3 mg/DL), also in high minerals increased levels of energy-protein increased serum P (4.6 vs 8.6 mg/DL). Ionized Ca followed the same interaction as serum Ca, increased levels of minerals in diets low and high energy-protein increased significantly serum ionized Ca (3.94 vs 4.94 and 3.58 vs 3.82 mg/DL, respectively). Serum Fe was increased ($P < .01$) when levels of energy-protein were increased in low and high mineral diets (180.8 vs 198.9 and 174.9 vs 255.1, respectively). Serum triglycerides were higher with increased levels of energy-protein only in high mineral diets (12.5 vs 17.2 µg/DL).

In the second sampling period (end of gestation), ewes fed high energy-protein diets were found to be higher ($P < .001$) in serum glucose (76.6 vs 57.7 mg/DL), balance = Na - (Cl + CO_2) (20.8 vs 15.2 meq/L), BUN (18.6 vs 8.4 mg/DL), BUN/creatine ratio (13.22 vs 6.56), total protein (7.18 vs 6.41 mg/DL), albumin (7.84 vs 3.30 g/DL), and globulin ($P < .05$) (3.36 vs 3.11 g/DL, respectively), than ewes fed low energy-protein diets. Serum Ca (9.18 vs 8.06 mg/DL), ionized Ca

(4.22 vs 3.43 mg/DL) and CO_2 (24.2 vs 18.9 meq/L) were found to be higher ($P < .001$) in low energy-protein diets than high energy-protein diets. There was an interaction ($P < .05$) between levels of energy-protein and levels of minerals only in total bilirubin (figure 3), increased mineral levels from low to high in low and high energy-protein diets decreased serum bilirubin levels (0.14 vs 0.11 and 0.13 vs 0.10 mg/DL, respectively).

In the third sampling period (end of lambing), ewes fed high energy-protein diets were found to be higher ($P < .001$) in serum balance = Na - (Cl + CO_2) (21.0 vs 15.9 meq/L), BUN (22.1 vs 13.4 mg/DL), BUN/creatine ratio (16.1 vs 11.3), total protein (7.20 vs 6.20 mg/DL), albumin (3.84 vs 3.06 g/DL), A/G ratio ($P < .01$) (1.12 vs 0.94), glucose (75.1 vs 62.0 mg/DL) and Fe ($P < .05$) (192.1 vs 160.7 µg/DL, respectively). Only serum CO_2 was higher ($P < .001$) in low-energy-protein diets vs high energy-protein diets (24.5 vs 18.4 meq/L); and higher ($P < .05$) in low mineral diets vs high mineral diets (21.46 vs 21.37 meq/L). There was no interaction ($P > .05$) between levels of energy-protein and levels of minerals in blood profiles in the third period. In second and third sampling periods levels of energy-protein were more important to take in consideration than previous level of minerals received for increasing blood profile levels in ewes.

Variance-pooled period analysis of metabolic blood profile (SMAC 25) in ewes fed four experimental diets are presented in table 35. Differences ($P < .01$) were found in the effect of period in serum Na, Cl, CO_2, balance = Na - (Cl + CO_2), BUN, creatine, BUN/creatine ratio, uric acid, Ca, P, total protein, albumin, ionized Ca, SAP, LDH,

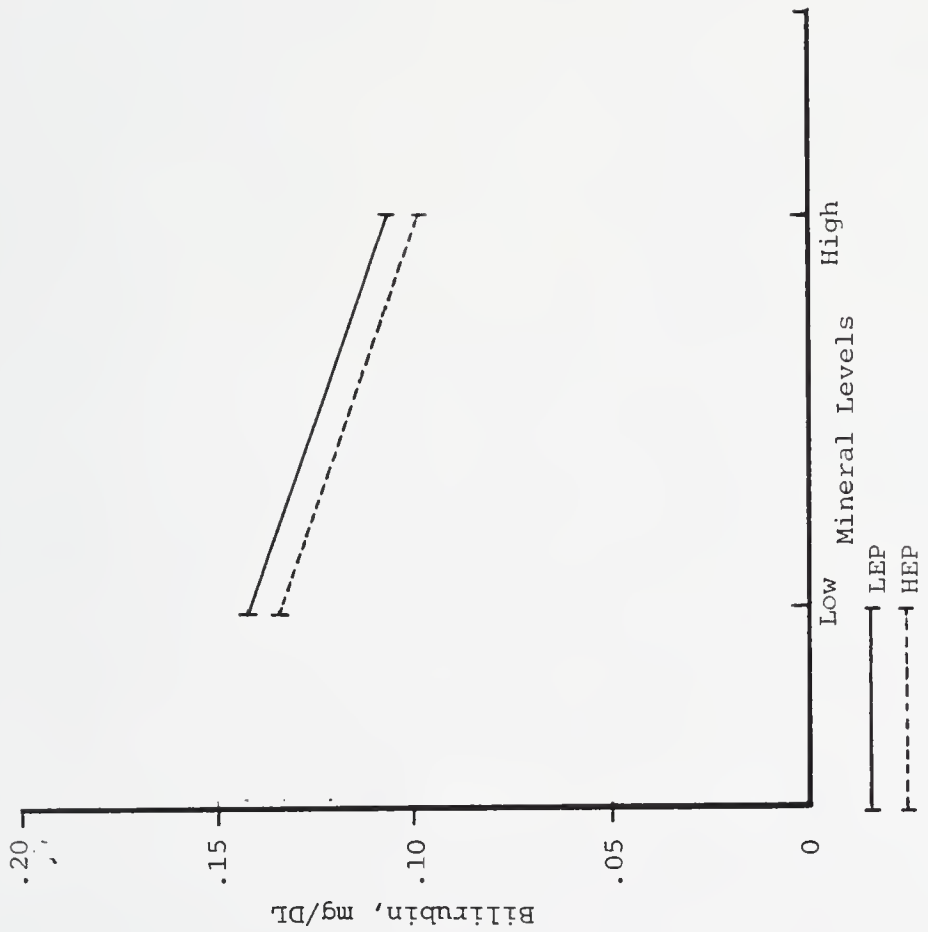

Figure 3. Interactions between levels of energy-protein and levels of minerals in serum bilirubin (SMAC 25) in ewes fed four experimental diets (period 2).

TABLE 36. ANALYSIS OF VARIANCE-POOLED PERIOD ANALYSIS OF METABOLIC BLOOD PROFILE (SMAC 25) IN EWES FED FOUR EXPERIMENTAL DIETS

SOURCE	PERIOD	ENERGY	MINERAL	ENERGY* MINERAL	EWES (DIET)	PERIOD* ENERGY	PERIOD* MINERAL	PERIOD*ENERGY MINERAL	ERROR
df	2	1	1	1	42	2	2	2	80
Glucose, mg/DL									
SS	112.22	5797.76	5.63	147.41	13885.60	673.77	1226.02	44.04	11207.89
MS	56.11	5797.76**	5.63	147.41	330.61**	336.89	613.01*	22.02	140.10
Na, meq/L									
SS	933.54	24.49	52.38	2.06	480.18	4.06	9.10	4.42	456.09
MS	466.77**	24.49	52.38*	2.06	11.43**	2.03	4.55	2.21	5.70
K, meq/L									
SS	1.69	1.48	1.19	0.09	17.13	0.06	0.16	0.09	26.39
MS	0.85	1.48	1.19	0.09	0.41	0.03	0.08	0.05	0.33
Chloride, meq/L									
SS	539.58	0.29	20.76	1.97	298.69	95.72	2.93	11.25	620.93
MS	269.79**	0.29	20.76	1.97	7.11	47.86**	1.47	5.63	7.76
CO_2, meq/L									
SS	92.23	736.72	0.14	7.22	514.25	86.37	0.49	63.55	484.93
MS	46.12**	736.72**	0.14	7.22	12.24**	43.19**	0.25	31.78**	6.06
Balance = Na-(Cl + CO_2), meq/L									
SS	348.19	1070.57	2.08	31.55	836.81	1.25	4.30	13.64	894.03
MS	174.10**	1070.57**	2.08	31.55	19.92**	0.63	2.15	6.82	11.18
BUN, mg/DL									
SS	586.08	4010.12	32.97	11.59	980.45	142.88	17.17	18.30	1012.65
MS	293.04**	4010.12**	32.97	11.59	23.34**	71.44**	8.59	9.15	12.66
Creatine, mg/DL									
SS	3.443	0.248	0.012	0.008	2.379	0.004	0.091	0.150	2.474
MS	1.722**	0.248*	0.012	0.008	0.057**	0.002	0.046	0.075	0.031
BUN/Creative ratio									
SS	1380.40	2170.05	30.68	10.93	633.96	373.26	30.97	1.02	1205.22
MS	690.2**	2170.05**	30.68	10.93	15.09	186.63**	15.49	0.51	15.07
Uric Acid, mg/DL									
SS	0.2684	0.0003	0.0212	0.0028	0.3075	0.1138	0.0188	0.0272	0.6316
MS	0.1342**	0.0003	0.0212	0.0028	0.0073	0.0569**	0.0094	0.0136	0.0079

TABLE 36–CONTINUED

SOURCE	PERIOD	ENERGY	MINERAL	ENERGY* MINERAL	EWES (DIET)	PERIOD* ENERGY	PERIOD* MINERAL	PERIOD*ENERGY MINERAL	ERROR
Ca, mg/DL									
SS	9.852	28.924	6.215	0.593	67.628	3.653	3.201	1.239	28.882
MS	4.926**	28.924**	6.215	0.593	1.610**	1.827**	1.601*	1.239*	0.361
P, mg/DL									
SS	182.713	80.892	32.737	0.003	298.222	24.017	12.851	13.738	200.249
MS	91.357**	80.892**	32.737*	0.003	7.101**	12.009**	6.426	6.869	2.503
Total Protein, g/DL									
SS	2.017	11.628	0.538	0.180	16.765	6.028	0.119	0.063	7.722
MS	1.009**	11.628**	0.538	0.180	0.399**	3.014**	0.060	0.032	0.097
Albumin, g/DL									
SS	1.127	10.338	0.055	0.020	7.670	1.121	0.177	0.006	3.906
MS	0.564**	10.338**	0.055	0.020	0.183**	0.561**	0.089	0.003	0.049
Globulin, g/DL									
SS	1.130	0.052	0.844	0.0045	18.213	1.828	0.384	0.534	21.433
MS	0.565	0.052	0.844	0.0045	0.434*	0.914*	0.192	0.267	0.268
A/G Ratio									
SS	0.1917	1.2006	0.0047	0.0139	3.7528	0.0156	0.0496	0.0761	3.3600
MS	0.0959	1.2006**	0.0047	0.0139	0.0894**	0.0078	0.0248	0.0381	0.0420
Ionized Ca, mg/DL									
SS	2.8226	13.4566	1.9332	0.0780	13.8526	0.4185	0.8843	0.5096	7.0176
MS	1.4113**	13.4566**	1.9332*	0.0780	0.3298**	0.2093	0.4422**	0.2548*	0.0877
Bilirubin, Total, mg/DL									
SS	0.0138	0.0038	0.0099	0.0014	0.0879	0.0092	0.0059	0.0007	0.1905
MS	0.0069	0.0038	0.0099*	0.0014	0.0021	0.0046	0.0030	0.0004	0.0024
Alkaline Phosphate, U/L									
SS	40395.37	351.51	30.07	17181.62	598796.67	8355.32	9045.44	3492.89	129702.98
MS	20197.69**	351.51	30.07	17181.62	14257.06**	4177.66	4522.72	1746.45	1621.29
LDH, U/L									
SS	781153.29	107357.25	61695.98	227037.85	2850843.04	221316.56	134962.10	92748.72	2873267.70
MS	390576.65**	107357.25	61695.98	227037.85	67877.21*	110658.28*	67481.05	46374.36	35915.85

TABLE 36—CONTINUED

SOURCE	PERIOD	ENERGY	MINERAL	ENERGY* MINERAL	EWES (DIET)	PERIOD* ENERGY	PERIOD* MINERAL	PERIOD*ENERGY MINERAL	ERROR
SGOT, U/L									
SS	28514.63	156.52	14158.66	29676.03	586090.89	37425.58	54336.83	35153.07	668232.85
MS	14257.32	156.52	14158.66	29676.03	13954.55	18712.80	27168.42*	17576.54	8352.91
SGPT, U/L									
SS	248.30	1.32	827.93	0.05	10054.10	359.31	144.34	3.79	17543.07
MS	124.15	1.32	827.93	0.05	239.38	179.66	72.17	1.90	219.29
Cholesterol, Total, mg/DL									
SS	3341.93	5068.85	1135.49	1403.03	95785.42	4913.75	2619.47	1328.90	167841.03
MS	1670.97	5068.86	1135.49	1403.03	2280.61	2456.88	1309.74	664.45	2098.01
Triglycerides, mg/DL									
SS	3569.02	3517.03	16.00	68.55	6798.57	3520.89	234.13	512.36	15255.15
MS	1784.51**	3517.03**	16.00	68.55	161.87	1760.45**	117.07	256.18	190.69
Fe, Serum µg/DL									
SS	35825.66	17586.88	13969.89	10527.77	127483.06	18461.78	1137.53	7623.04	115863.38
MS	17912.83**	17586.88	13979.89	10527.77	3035.31**	9230.89**	568.77	3811.52	1448.29

* Significant at (P < .05).

** Significant at (P < .01).

triglycerides and Fe. Differences ($P < .01$) also were found in the effect of level of energy-protein in serum glucose, CO_2, balance = Na - (Cl + CO_2), BUN, BUN/creatine ratio, Ca, P, total protein, albumin, A/G ratio, ionized Ca, triglycerides and creatine ($P < .01$). Differences ($P < .05$) were found in the effect of level of minerals only in serum Na, P, ionized Ca and bilirubin, total. No interactions ($P < .05$) between levels of energy-protein and levels of minerals were found. Interaction period x levels of energy-protein ($P < .01$) were found in serum Cl, CO_2, BUN, BUN/creatine ratio, uric acid, Ca, P, total protein, albumin, triglycerides, Fe and globulin and LDH ($P < .05$). Also, significant interactions ($P < .05$) between period x minerals were found in serum glucose, Ca, SGOT and ionized Ca ($P < .01$). Significant interactions ($P < .01$) between period x energy-protein x mineral were found only in serum CO_2 and Ca, ionized Ca ($P < .05$) in the pooled periods analysis of variance of metabolic blood profile in ewes. There was a significant effect of period (increased) in serum Na (142.9, 145.1 and 149.3 meq/L); Cl (104.8, 105.5 and 109.5 meq/L); balance = Na - (Cl + CO_2) (14.7, 18.0 and 18.4 meq/L) and P (7.5, 8.2 and 10.4 mg/DL) in periods 1, 2 and 3, respectively (Figure 4). There was an effect of period (decreased in CO_2 (23.3, 21.6 and 21.5 meq/L); Ca (8.8, 8.6 and 8.1 mg/DL); ionized Ca (4.0, 3.8 and 3.6 mg/DL) and SAP (215.2, 191.8 and 176.8 U/L) for periods 1, 2 and 3, respectively. Serum creatine (1.0, 1.4 and 1.3 mg/DL); uric acid (0.12, 0.31 and 0.20 mg/DL); total protein (6.48, 6.79 and 6.68 g/DL);

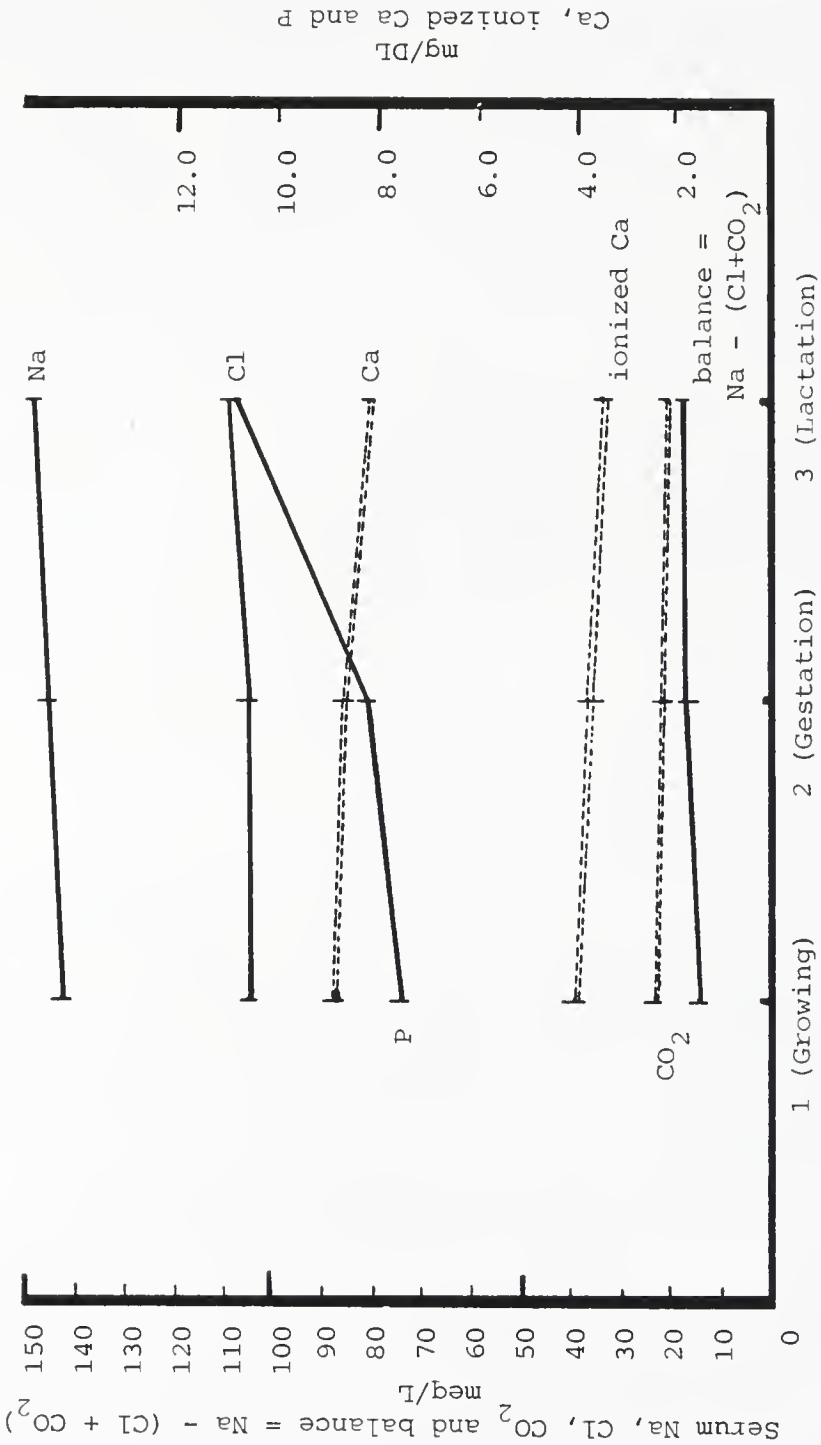

Figure 4. Effect of period in metabolic blood profiles (SMAC 25).

albumin (3.34, 3.57 and 3.43 g/DL); triglycerides (15.6, 28.6 and 22.5 mg/DL) and Fe (200.7, 213.8 and 174.6 mg/DL) were higher in second period (end of gestation) versus first (end of growing) and third (end of lambing), respectively. There was no significant ($P > .05$) carry-over effect of period in serum glucose, K, globulin, A/G ratio, bilirubin, SGOT, SGPT and cholesterol.

Metabolic blood profiles in weaned lambs

Means of blood metabolic profiles (SMAC 25) and Se in weaned lambs from ewes fed four experimental diets are presented in table 37, while appendix table 77 shows analysis of variance and mean squares for weaning lambs. There was an interaction ($P < .05$) between female and male only in serum SGPT. There was an interaction ($P < .05$) between high and low energy-protein diets in serum BUN, BUN/creatine ratio and Fe. Also, there was an interaction ($P < .05$) between sex and levels of energy-protein in female lambs increased SPOT (11.63 vs 11.83 U/L). Significant interaction ($P < .05$) between previous high minerals for ewe diets was found only in serum Cl and Fe; there were weaning lambs from ewes fed low mineral diets only (104.1 meq/L and 107 µg/DL, respectively). Interaction ($P < .05$) between sex and level of minerals was found in serum BUN, total protein and Fe; increased minerals from low to high in female lambs increased serum BUN and total protein (14.5 vs 18.0 mg/DL and 4.95 vs 6.18 mg/DL) and decreased serum Fe (216.0 vs 132.5 µg/DL). There were no lambs representing the diet fed to ewes high in minerals. Serum BUN, total protein and Fe in weaning lambs from ewes fed low mineral diets were

TABLE 37. MEANS, STANDARD ERRORS OF BLOOD METABOLIC PROFILES (SMAC 25) AND SELENIUM IN WEANING LAMBS FROM EWES FED FOUR EXPERIMENTAL DIETS

Variable	DIET 1		DIET 2		DIET 3		DIET 4	
	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error
Glucose, mg/DL	74.20	4.18	57.00	19.00	67.50	11.50	69.17	0.98
Na, meq/L	146.40	0.68	147.50	2.50	145.50	0.50	145.67	1.20
K, meq/L	5.40	0.15	5.55	0.05	5.20	0.00	5.42	0.09
Chloride, meq/L	104.40	0.81	108.50	0.50	104.00	2.00	105.17	0.65
CO_2, meq/L	21.00	1.30	19.50	1.50	23.00	3.00	20.83	1.05
Balance = Na - ($Cl+CO_2$) meq/L	21.00	1.48	19.50	3.50	18.50	0.50	19.67	1.78
BUN, mg/DL	10.20	0.66	11.00	3.00	21.00	2.00	20.50	2.50
Creatine, mg/DL	0.80	0.03	0.75	0.15	0.80	0.00	0.73	0.05
BUN/Creatine ratio	13.00	1.14	16.00	7.00	26.50	2.50	27.50	2.19
Uric Acid, mg/DL	0.20	0.00	0.20	0.00	0.20	0.00	0.20	0.00
Ca, mg/DL	9.70	0.22	9.55	0.45	10.05	0.15	10.35	0.22
P, mg/DL	10.52	0.66	9.45	0.55	9.30	0.10	8.50	0.70
Total Protein, g/DL	6.05	0.08	5.95	0.15	6.40	0.50	6.20	0.14
Albumin, g/DL	3.14	0.09	3.45	0.05	3.45	0.25	3.33	0.16
Globulin, g/DL	2.90	0.07	2.50	0.20	2.95	0.25	2.87	0.10
A/G Ratio	1.04	0.05	1.35	0.15	1.10	0.00	1.15	0.09
Ionized Ca, mg/DL	4.62	0.10	4.60	0.30	4.65	0.15	4.88	0.05
Bilirubin, Total, mg/DL	0.12	0.02	0.10	0.00	0.10	0.00	0.10	0.00
Alkaline Phosphatase U/L	315.20	48.01	278.00	95.00	466.50	137.50	342.00	21.80
LDH, U/L	350.80	41.60	293.50	106.50	291.50	21.50	325.67	13.37
SGOT, U/L	99.00	17.45	100.00	18.00	71.00	9.00	112.83	36.60
SGPT, U/L	22.60	9.27	9.50	0.50	8.50	3.50	16.33	5.94
Cholesterol, Total, mg/DL	100.40	6.86	100.00	10.00	87.50	10.50	105.23	8.05
Triglycerides, mg/DL	44.40	6.42	30.50	1.50	25.00	9.00	39.17	8.49
Fe, µg/DL	152.00	18.63	126.00	24.00	204.00	60.50	152.83	14.36
Se, µg/ml	0.137	0.01	0.09	0.004	-	-	0.10	0.005

[a] Means based on five observations on diet 1, except Se with four observations; two observations on diets 2 and 3, and six observations on diet 4, except Se with three.

17.0 mg/DL, 6.50 mg/DL and 188.0 µg/DL, respectively. No significant ($P < .05$) interaction of energy-protein x minerals and sex x energy-protein-minerals was found in metabolic blood parameters (SMAC 25).

Summary

A sequential multiple analyzer with computer (SMAC 25) was used to obtain ovine blood profiles containing values for glucose, sodium (Na), potassium (K), chloride (Cl), carbon dioxide (CO_2), balance = Na - (Cl + CO_2), blood urea nitrogen (BUN), creatine, BUN/creatine ratio, uric acid, calcium (Ca), phosphorus (P), total protein, albumin, globulin, A/G ratio, ionized Ca, bilirubin total, alkaline phosphatase (SAP), lactic dehydrogenase (LDH), serum glutamic-oxalacetic transaminase (SGOT), serum glutamic-pyruvate transaminase (SGPT), cholesterol total, triglycerides and Fe in serum. The principal objective of this experiment was to compare age and physiological status (wether lambs, pregnant ewes and lactating and nursing lambs) on metabolic blood profile, considered the effect of two dietary levels of energy-protein (low = .8 x maintenance, high = 1.8 x maintenance) and two levels of minerals (low and high) on these blood parameters and determine carry-over effect of high dietary minerals as effected by high and low dietary energy-protein. Samples were taken from eleven wether lambs at the end of the second trial when dietary mineral concentrations had been reduced (low minerals) with two levels of energy-protein from forty-five ewes in three periods (end of growing, gestation and lambing periods), and from fourteen nursing lambs at the end of weaning time.

In the wether experiment, serum K was higher ($P < .01$) in animals fed diets LEP + LM versus animals fed diets HEP + LM (5.07 vs 4.04 meq/L). Albumin/globulin ratio, triglycerides ($P < .01$) and albumin ($P < .05$) were higher in diets HEP + LM versus diets LEP + LM (1.34 vs 1.07, 56.80 vs 28.30 mg/DL, and 3.48 vs 3.20 g/DL, respectively).

In the first period of the ewe experiment (end of growing period), ewes fed high energy-protein diets (HEP + LM, HEP + HM) were found to be higher ($P < .05$) in serum glucose, balance = Na - (Cl + CO_2), BUN, creatine, BUN/creatine ratio, P, albumin, A/G ratio, LDH, SGOT and Fe than ewes fed low energy-protein diets LEP + LM, LEP + HM (72.8 vs 64.6 mg/DL, 17.7 vs 11.7 meq/L, 25.1 vs 11.0 mg/DL, 1.04 vs .96 mg/DL, 24.2 vs 11.5, 8.9 vs 6.0 mg/DL, 3.52 vs 3.17 g/DL, 1.15 vs .93, 610 vs 519 U/L, 119 vs 89 U/L, and 227.0 vs 177.8 µg/DL, respectively). In contrast, serum CO_2, Cl, uric acid, Ca, globulin and ionized Ca were found to be higher ($P < .05$) in ewes fed low energy-protein diets versus ewes fed high energy-protein diets (24.6 vs 22.0 meq/L, 105.9 vs 103.5 meq/L and .26 vs .19 mg/DL, respectively). Serum from ewes fed high mineral diets, HM + LEP and HM + HEP, were found to be higher ($P < .05$) in Ca, ionized Ca and Fe than ewes fed low mineral diets, LM + LEP and LM + HEP (9.2 vs 8.3 mg/DL, 4.23 vs 3.76 mg/DL and 215.0 vs 189.8 µg/DL, respectively). In contrast, serum glucose, Na and P were found to be higher ($P < .05$) in ewes fed low mineral diets versus ewes fed high mineral diets (72.7 vs 64.7 mg/DL, 143.9 vs 141.8 meq/L, and 8.4 vs 6.6 mg/DL, respectively). Also, there was an interaction ($P < .05$) between levels of energy-protein and levels of minerals in serum creatine, Ca, ionized Ca, triglycerides, P and Fe.

Serum Ca was increased ($P < .05$) when levels of minerals were increased either in low or high energy-protein diets (8.7 vs 10.1 and 7.8 vs 8.3 mg/DL, respectively). Also serum P and Fe increased ($P < .05$) in low and high mineral diets with increased energy-protein levels (7.4 vs 9.3, 4.6 vs 8.6 mg/DL and 180.8 vs 198.9, 174.9 vs 255.1 µg/DL, respectively).

In the second period, ewes fed high energy-protein diets were found to be higher ($P < .05$) in serum glucose (76.6 vs 57.7 mg/DL), balance = Na - (Cl + CO_2) (20.8 vs 15.2 meq/L), BUN (18.6 vs 8.4 mg/DL), BUN/creatine ratio (13.22 vs 6.56), total protein (7.18 vs 6.41 mg/DL), albumin (7.84 vs 3.30 g/DL), and globulin (3.36 vs 3.11 g/DL), respectively, than ewes fed low energy-protein diets. In contrast, serum Ca (9.18 vs 8.05 mg/DL), ionized Ca (24.2 vs 18.9 meq/L), and CO_2 (4.22 vs 3.43 mg/DL) were found higher ($P < .001$) in low energy-protein diets versus high energy-protein diets. Serum bilirubin was decreased with increased mineral levels in low and high energy-protein diets (.14 vs .11 and .13 vs .10 mg/DL, respectively).

In the third period, ewes fed high energy-protein diets were found to be higher ($P < .001$) in serum balance = Na - (Cl + CO_2) (21.0 vs 15.9 meq/L), BUN (22.1 vs 13.4 mg/DL), BUN/creatine ratio (16.1 vs 11.3), total protein (7.20 vs 6.20 mg/DL), albumin (3.84 vs 3.06 g/DL), A/G ratio ($P < .01$) (1.12 vs .94), glucose (75.1 vs 62.0 mg/DL) and Fe ($P < .05$) (192.1 vs 160.7 µg/DL), respectively.

In weaned lambs, there was a significant interaction between female and male (sex effect) only in SGPT; also an interaction was found ($P < .05$) between high and low energy diets in serum BUN,

BUN/creatine ratio and Fe. There was an interaction between sex and level of energy-protein only in serum CO_2 and SGPT. An interaction ($P < .05$) between levels of minerals was found in serum Cl and Fe. Also, interactions ($P < .05$) between sex and level of minerals were found in serum BUN, total protein and Fe.

CHAPTER VII
GENERAL SUMMARY AND CONCLUSIONS

Low ruminant productivity in the tropics is due to many limitations, the most apparent being the low feed intake and poor nutrition (protein, energy, minerals) which consequently result in poor growth and reproductive rates. Fluctuation of nutrient content of the pasture results in a particular pattern of growth rate of animals on native grasses; that is, rapid growth in the rainy season followed by loss of body weight during the dry season (Van Niekerk, 1974). During the dry season, energy and/or protein deficiencies limited cattle production but during the rainy season, mineral deficiencies may be the major factor which affects rate of cattle production because protein and digestible energy contents of growing grasses are adequate to meet their requirements for maintenance and production (McDowell, 1976).

Increased incidences of mineral deficiencies during the wet season are less related to forage mineral concentrations than to the greatly increased requirements for these elements by the grazing animals (Correa, 1957; Van Niekerk, 1974; McDowell, 1976). Adequate intake of forages by cattle is essential in meeting mineral requirements. Factors which greatly reduce forage intake, such as low protein (< 7.0%) content and increased maturity, lignification and stem--leaf ratios all reduce the total mineral consumed (McDowell, 1976).

Effect of energy-protein on mineral supplies generally is unknown; it is possible to supplement for minerals during a short period to carry-over to periods when supplies are inadequate. Cattle do, however, survive such periods of undernutrition by utilizing their energy conservation mechanism. They are able to mobilize and deplete body tissue reserves during periods of food scarcity and to replenish the reserves when food is readily available. Therefore, a multitude of factors influence the productivity of cattle in tropical areas, particularly inadequate nutrition during the long dry periods (Sanchez, 1976). Thus, the objectives of this study were to investigate the effect of different energy and protein levels on mineral utilization by the animal (Ca, P and Mg) and to compare to mineral levels (high and low) on mineral storage and long-term carry-over effects in ruminants.

In the first experiment twelve Florida native crossbreed wether lambs were randomly assigned to two treatment groups, where two levels of energy (low = .8 x maintenance; high = 1.8 x maintenance) and two levels of minerals (low = around maintenance; high = 2 x 30 times maintenance requirements) were studied as they affected Ca, P and Mg retention. In the first trial, the animals were fed a semi-purified diet high in minerals with two levels of energy-protein. Wether lambs were fed the two experimental diets for three months before being placed in metabolism cages for retention studies. In the second trial, dietary mineral concentrations were reduced and the same animals were fed again for another three months with a diet low in minerals with two levels of energy-protein. Following the three-month period, animals were placed in metabolism cages for retention studies.

Wethers fed high energy-protein diets with either high or low mineral concentrations had greater gains than diets low in energy-protein. High energy-protein diets (1.8 x maintenance requirements) increased wethers' average daily gain 106.2 g/animal, while wethers fed low energy-protein diets (.8 x maintenance requirements) averaged only 12.4 g/d/animal. Levels of energy-protein and increased levels of dietary minerals did not affect serum mineral concentrations; it seems through homeostatic mechanisms that the animal mobilizes minerals from body reserves to maintain normal serum concentrations. High dietary energy-protein concentrations in the presence of low minerals apparently depressed liver Fe, Cu and Co. Wethers fed high mineral diets had higher ($P < .05$) concentrations of liver Mn, Co and Zn. All animals in the second trial (LM + LEP and LM + HEP) contained liver Se levels above the .25 ppm reported by McDowell et al. (1978) as normal for grazing cattle. The low energy-protein diet with low minerals increased ($P < .05$) kidney Fe concentration compared to the high energy-protein with low minerals (641.0 vs 165.2 ppm). No differences ($P > .05$) were found for any other tissue mineral (heart, spleen and muscle).

The diets high in energy-protein with low minerals were higher ($P < .05$) in wool P and Na (171 vs 117 ppm and 1146 vs 795 ppm, respectively); Mg also was higher ($P < .01$) (68 vs 43 ppm) in wethers fed diet LM + HEP versus LM + LEP, respectively.

For trial 1, differences ($P < .05$) were found between diets 2 and 4 (high minerals) only in Mg, expressed both as percent dry, fat-free bone (.59 vs .71%) and percent ash (.97 vs 1.19). In trial 2, Mg expressed

as % bone ash was higher ($P < .01$) in diet 3 (LM + HEP) than diet 1 (LM + LEP) (1.16 vs .89%). No differences ($P > .05$) were found between the two experimental diets (LM + LEP, LM + HEP) in any metacarpal bone parameters.

In trial 1 (high minerals), Ca and P retention was less ($P < .05$) with diets low in energy-protein (1.41 vs 2.74 g/d Ca and 2.57 vs 4.53 g/d P). Also, Mg retention was less ($P < .01$) in wethers fed the low energy-protein diet (1.32 vs 2.85 g/d). In trial 2, significant differences ($P < .01$) were found between diets 1 and 3 (LM + LEP, LM + HEP) in Ca, P and Mg retention (.72 vs 1.51 g/d, 1.32 vs 2.77 g/d, and 1.12 vs .84 g/d, respectively). In the present experiment, the major route for Ca and P excretion was feces and for Mg excretion, urine. Urinary Mg excretion was particularly higher in wethers fed diets which were high in minerals (diets 2 and 4) in comparison with wethers fed diets low in minerals (diets 1 and 3).

In the second experiment effects of two levels of energy-protein on sheep mineral status (macro elements) and two levels of minerals (high and low) on mineral storage and long-term carry-over effects in sheep were studied. Forty-eight Rambouillet crossbreed ewe lambs were randomly assigned to four experimental diets in a 2 x 2 factorial arrangement of treatment groups. The duration of the experiment was 18 months, divided in four periods: growing, breeding, gestation-parturition and lactation. Treatments with high minerals were administered for only four months of the growing period; ewes were fed low mineral diets with high and low energy-protein levels for the remainder of the trial.

During this 18-month experiment, levels of energy-protein were more important than levels of minerals for animal performance. No carry-over effects were observed in hematocrit, Hb and serum Ca, P, Mg, Na and K of ewes fed for four months the original experimental diets which are high in mineral levels. Serum P was increased from growing through lactation periods in ewes fed high minerals with low energy-protein diets (HM + LEP) 5.2, 8.3, 8.6 and 9.3 mg/100 ml, respectively. In the growing period bone rib P was higher in ewes fed high minerals with low and high (HM + LEP, HM + HEP) energy-protein diets than ewes fed low mineral diets (LM + LEP, LM + HEP), expressed as % of dry, fat-free bone, % of bone ash and mg/cc (11.35 and 11.55 vs 9.22 and 10.77%; 18.45 and 18.75 vs 15.02 and 17.33%; 195 and 222 vs 174 and 155 mg/cc, respectively). For the breeding period, bone P continued to be higher in high versus low mineral diets (11.60 and 11.10 vs 9.50 and 1.00%; 18.90 and 19.90 vs 18.0 and 18.60%; 206 and 148 vs 159 and 126 mg/cc, respectively); thus feeding the high minerals resulted in a carry-over effect for bone P in period 2, but for period 3 (gestation-parturition) no carry-over effect of P was observed.

No differences ($P < .05$) were found in milk and colostrum Ca, P, Mg, Na and K between high and low energy-protein dietary levels. Differences ($P < .05$) between levels of minerals were found only in milk Mg concentrations. No sex differences ($P > .05$) were found between male and female lambs in body weights (at birth and weaned). In all lambs, hematocrit and Hb concentrations were higher ($P < .05$) in newborn versus the lactation period (44.6 vs 32.5% and 14.7 vs

11.3%, respectively). In contrast, bone tail mineral concentration means (Ca, P and Mg) for weaned lambs were higher than in newborn lambs, particularly as expressed as percent of ash and Ca and P as percent of dry, fat-free bone.

In the third period, experimental effects of two levels of energy-protein on mineral status (trace elements) and two levels of minerals on mineral storage and long-term carry-over effects in sheep were studied. No carry-over effects were observed in serum Fe, Cu, Zn and Se in ewes fed the original experimental diets (high minerals) for four months. In the growing period, liver Mo concentrations were higher in ewes fed high versus low mineral diets with two energy-protein levels (low and high) (3.74, 4.79 vs 2.83, 3.65 ppm). In the breeding period, liver Mo was increased in ewes fed high mineral diets with low and high energy-protein levels (8.40 and 6.44 ppm). In period 3 (gestation-parturition), liver Mo concentrations were increased from 8.40 to 9.95 ppm only in animals receiving high minerals with high energy-protein diets, and decreased from 6.44 to 4.57 in those receiving high minerals with low energy-protein diets. Thus, carry-over effects were observed in liver Mo concentrations of ewes fed high minerals with low energy-protein diets until the breeding period and in ewes fed high minerals with high energy-protein diets until the gestation-parturition period. No carry-over effects were observed in liver Fe, Cu, Zn, Mn and Se concentrations.

Period x energy-protein level interactions were found only in liver Cu concentrations. Period x mineral interactions were found in liver Cu and Mo concentrations. Differences ($P < .05$) were found

between dietary energy-protein levels (high and low) in wool Zn concentrations (1004 vs 892 µg/g). Milk has higher Fe and Mn concentrations than colostrum; in contrast, Cu, Zn and Se were higher in colostrum than milk. Serum Fe, Cu and Zn decreased from newborn to weaned lambs.

In the fourth experiment, a sequential multiple analyzer with computer (SMAC 25) was used to obtain ovine blood profiles containing values for glucose, sodium (Na), potassium (K), chloride (Cl), carbon dioxide (CO_2), balance = Na - (Cl + CO_2), phosphorus (P), total protein, albumin, globulin, A/G ratio, ionized Ca, bilirubin total, alkaline phosphatase (SAP), lactic dehydrogenase (LDH), serum glutamic-oxalacetic transaminase (SGOT), serum glutamic-pyruvate transaminase (SGPT), cholesterol total, triglycerides and Fe in serum. The principal objective of this experiment was to compare age and physiological status (wether lambs, pregnant ewes and lactating and nursing lambs) on metabolic blood profiles, and consider the effect of two dietary levels of energy-protein and two levels of minerals on these blood parameters.

In wether lambs, serum K was higher ($P < .01$) in animals fed low mineral with low energy-protein diets (LM + LEP) versus animals fed low minerals with high energy-protein diets (5.07 vs 4.04 meq/L). Albumin/globulin ratio, triglycerides ($P < .01$) and albumin ($P < .05$) were higher in low mineral, high energy-protein versus low mineral, low energy-protein diets (1.34 vs 1.07, 56.80 vs 28.0 mg/DL, and 3.48 vs 3.20 g/DL, respectively).

In the first period of the ewe experiment (end of growing period) ewes fed high energy-protein diets with low and high minerals were found to be higher ($P < .05$) in serum glucose, balance = Na - (Cl + CO_2), BUN, creatine, BUN/creatine ratio, P, albumin, A/G ratio, LDH, SGOT and Fe than ewes fed low energy-protein diets with low and high minerals (72.8 vs 64.6 mg/DL, 17.7 vs 11.7 meq/L, 25.1 vs 11.0 mg/DL, 1.04 vs .96 mg/DL, 24.2 vs 11.5, 8.9 vs 6.0 mg/DL, 3.52 vs 3.17 g/DL, 1.15 vs .93, 610 vs 519 U/L, 119 vs 89 U/L and 227.0 vs 177.8 µg/DL, respectively). In contrast, serum CO_2, Cl, uric acid, Ca, globulin and ionized Ca were found to be higher ($P < .05$) in ewes fed low energy-protein versus high energy-protein diets (24.6 vs 22.0 meq/L, 105.9 vs 103.5 meq/L and .26 vs .19 mg/DL, respectively). Serum from ewes fed high mineral diets were found to be higher ($P < .05$) in Ca, ionized Ca and Fe than ewes fed low mineral diets (9.2 vs 8.3 mg/DL, 4.23 vs 3.76 mg/DL and 215.0 vs 189.8 g/DL, respectively). In contrast, serum glucose, Na and P were found to be higher ($P < .05$) in low mineral versus high mineral diets (72.7 vs 64.7 mg/DL, 143.9 vs 141.8 meq/L and 8.4 vs 6.6 mg/DL, respectively).

In the second period, serum Ca, ionized Ca and CO_2 were found higher ($P < .001$) in low energy-protein versus high energy-protein diets. In the third period, ewes fed high energy-protein diets were found to be higher ($P < .001$) in serum balance = Na - (Cl + CO_2), BUN, BUN/creatine ratio, total protein, albumin, A/G ratio, glucose and Fe than ewes fed low energy-protein levels. In weaned lambs, there was a significant interaction between female and male (sex effect)

only in SGPT. Also, an interaction ($P < .05$) between high and low energy-protein diets was found in serum BUN, BUN/creatine ratio and Fe.

In conclusion, no carry-over effects were found in serum metabolic blood profiles (SMAC 25) of ewes fed high mineral levels for a four-month period.

APPENDIX

TABLE 38. CHEMICAL COMPOSITION OF EXPERIMENTAL DIETS (DRY BASIS)

	PROXIMATE ANALYSES *						MINERAL ANALYSES *										
Diets[1]	Dry Matter %	Ash %	Crude Fiber %	E.E. %	Crude Protein %	NFE %	Ca %	P %	Mg %	Na %	K %	Fe ppm	Cu ppm	Zn ppm	Mn ppm	Co ppm	Se ppm
1. LEP+LM	91.40	3.42	28.50	1.19	8.48	49.80	0.205	0.210	0.160	0.388	1.030	72.00	5.74	81.20	26.20	0.44	0.12
2. LEP+HM	90.10	6.65	29.53	0.76	6.21	46.90	0.475	0.471	0.295	0.640	1.080	136.80	11.62	235.40	79.90	1.22	0.19
3. HEP+LM	90.05	3.74	5.82	3.07	14.74	62.70	0.207	0.422	0.165	0.470	0.870	102.00	6.78	64.80	18.90	0.83	0.51
4. HEP+HM	89.60	6.93	5.04	1.38	14.45	61.80	0.446	0.609	0.443	0.923	1.200	139.20	12.80	281.10	83.60	1.03	1.20

[1] Diets identification.

1. Low Energy, Low Protein, Low Minerals.
2. Low Energy, Low Protein, High Minerals.
3. High Energy, High Protein, Low Minerals.
4. High Energy, High Protein, High Minerals.

* Means based on eight analyses (two per experimental period).

TABLE 39. EFFECT OF ENERGY-PROTEIN ON BODY WEIGHT, SERUM MINERALS, HEMOGLOBIN AND HEMATOCRIT IN SHEEP

EXPERIMENT I, TRIALS 1 AND 2

	Wether No	BODY WEIGHT[a] (KG)				BLOOD		SERUM MINERALS								
		1st	2nd	3rd	4th	Hemoglobin g/100 ml	Hematocrit %	Ca mg/100 ml	P mg/100 ml	Mg mg/100 ml	Na µg/ml	K µg/ml	Fe µg/ml	Cu µg/ml	Zn µg/ml	Se µg/ml
Trial 1	1	48.6	45.40	41.8	40.9	12.0	55	16.5	5.2	3.2	3058	200	2.29	1.15	1.14	-
	2	39.5	42.30	43.2	43.2	12.0	54	13.6	5.2	3.7	2950	174	2.29	1.46	0.76	0.155
	3	53.2	50.40	53.6	50.0	12.3	58	14.0	5.2	3.4	3129	152	1.43	0.23	0.86	-
Diet 2	4	53.6	57.30	60.0	55.4	10.7	46	13.8	7.2	3.3	3058	163	2.29	1.00	1.19	0.151
	5	49.10	54.50	54.1	50.9	11.0	50	9.5	5.0	2.5	2914	163	2.57	1.08	1.32	0.151
	6	42.70	44.10	44.5	45.4	12.0	55	12.4	6.2	2.4	3237	163	1.71	0.92	1.32	0.173
	7	43.2	48.60	52.7	56.8	9.6	42	12.9	7.4	3.8	3201	185	3.14	1.08	1.41	0.224
	8	50.4	55.90	60.0	61.8	11.6	51	10.5	7.4	2.1	3022	174	2.14	1.08	1.30	0.180
Diet 4	9	39.5	42.70	45.4	43.6	9.1	35	12.1	9.7	4.2	3237	174	2.00	1.46	1.41	0.227
	10	39.10	44.10	45.6	48.6	12.0	55	14.6	9.6	4.2	3201	261	2.29	1.08	1.03	0.198
Trial 2	1	42.7	40.90	40.9	-	11.9	50	11.9	5.2	2.2	3201	176	2.14	0.77	1.38	0.133
	2	61.8	62.30	60.0	-	14.1	56	12.1	7.9	3.2	3165	154	2.00	0.85	1.38	0.107
	3	50.9	52.70	54.5	-	14.4	45	13.2	4.6	3.4	2986	143	2.00	0.77	1.14	-
Diet 1	4	44.1	48.20	50.0	-	12.9	59	13.7	6.0	3.1	3094	143	1.86	0.69	1.24	-
	5	52.3	51.80	48.2	-	9.6	40	13.9	7.7	3.2	2950	187	2.57	1.08	1.16	0.113
	6	46.4	49.50	49.1	-	11.6	50	13.9	6.0	3.3	2950	165	0.86	0.77	3.81	-
	7	60.4	68.20	70.0	-	13.7	54	11.3	6.8	4.2	3022	165	3.14	1.08	1.65	-
	8	48.6	51.80	52.7	-	15.0	61	11.0	6.2	2.4	3129	187	2.57	0.38	1.41	-
Diet 3	9	52.7	58.20	57.3	-	11.3	46	11.8	7.7	4.0	3022	176	2.29	0.85	1.51	0.147
	10	65.0	70.90	72.7	-	10.9	43	14.1	7.2	4.5	2842	143	2.29	1.00	1.54	0.113
	11	68.2	71.40	74.5	-	13.7	55	10.9	8.3	2.7	3129	165	2.14	0.92	1.84	-

[a] Body weight measured monthly.

TABLE 40. EFFECT OF ENERGY-PROTEIN ON TISSUE MINERAL COMPOSITION IN SHEEP

EXPERIMENT I, TRIAL 2

		MINERAL CONCENTRATION, PPM DRY MATTER BASIS												
		KIDNEY						HEART						
Diet	Wether No	Fe	Cu	Zn	Mn	Co	Mo	Fe	Cu	Zn	Mn	Co	Mo	Se
1	1	989	28	97	3.20	0.14	2.03	308	7	57	1.11	0.02	0.07	-
	2	175	13	61	1.64	0.19	3.20	104	-	42	0.57	0.13	0.29	-
	3	771	8	46	1.11	0.23	1.71	171	24	83	0.97	0.09	0.22	-
	4	289	38	79	3.98	0.15	4.74	149	19	59	0.95	0.04	0.26	-
	5	905	12	75	1.87	0.14	1.96	148	18	59	0.96	0.05	0.18	-
	6	717	13	74	2.59	0.09	2.79	84	7	34	0.45	0.08	0.24	0.880
3	7	103	15	58	1.91	0.12	2.33	112	13	48	0.71	0.07	0.21	1.126
	8	219	25	80	3.19	0.24	2.34	201	21	75	1.35	0.14	0.26	-
	9	88	10	52	1.61	0.24	2.36	161	19	53	1.44	0.07	0.18	-
	10	256	12	70	1.81	0.07	2.47	138	16	55	0.84	0.09	0.18	-
	11	160	17	77	2.48	0.17	2.68	159	15	59	1.07	0.04	0.18	1.180
		SPLEEN						MUSCLE						
Diet	Wether No	Fe	Cu	Zn	Mn	Co	Mo	Fe	Cu	Zn	Mn	Co	Mo	Se
1	1	740	6	59	0.75	0.08	0.32	75	11	79	1.43	0.06	0.08	-
	2	726	5	56	0.43	0.07	0.19	58	5	69	0.53	0.02	0.03	-
	3	301	6	83	0.80	0.05	0.40	94	16	85	1.50	0.07	0.11	-
	4	948	12	89	1.70	0.12	0.62	80	5	91	0.43	0.02	0.09	-
	5	340	18	343	0.83	0.03	0.31	93	21	79	1.99	0.01	0.08	-
	6	224	9	46	0.45	0.02	0.20	76	2	123	3.12	0.02	0.08	-

TABLE 40—CONTINUED

Diet	Wether No	Fe	Cu	Zn	Mn	Co	Mo	Fe	Cu	Zn	Mn	Co	Mo	Se
3	7	360	7	73	0.71	0.12	0.30	58	5	120	0.46	0.01	0.10	-
	8	307	8	95	0.93	0.05	0.33	98	28	211	2.66	0.01	0.06	-
	9	-	-	-	-	0.08	0.27	74	14	101	0.36	0.02	0.05	-
	10	906	6	95	1.07	0.09	0.36	58	11	76	1.39	0.01	0.04	-
	11	947	5	107	0.84	0.09	0.39	64	8	97	1.28	0.80	0.09	-

TABLE 41. EFFECT OF ENERGY-PROTEIN ON LIVER MINERAL COMPOSITION IN SHEEP

EXPERIMENT I, TRIALS 1 AND 2

	MINERAL CONCENTRATION, PPM, DRY MATTER BASIS							
	Wether No.	Fe	Cu	Zn	Mn	Co	Mo	Se
Trial 1	1	368	221	111	8.16	0.34	5.40	-
	2	284	174	101	6.64	0.48	8.00	-
	3	525	261	370	17.64	1.14	5.01	-
Diet 2	4	367	68	36	10.15	0.90	7.43	-
	5	262	221	472	6.40	0.34	4.82	-
	6	331	971	356	10.83	0.35	3.33	-
	7	-	-	-	-	-	-	-
	8	302	65	81	8.45	0.34	2.39	-
Diet 4	9	203	230	109	7.16	0.37	4.81	-
	10	985	308	406	6.33	0.92	4.02	-
Trial 2	1	318	288	74	4.55	0.28	4.94	0.100
	2	220	52	62	2.74	0.29	4.12	0.604
	3	173	168	68	3.69	0.23	5.07	0.267
Diet 1	4	289	147	79	4.97	0.22	2.12	0.650
	5	655	248	99	3.83	0.16	2.97	0.509
	6	379	385	92	6.50	0.23	4.57	0.556
	7	104	61	53	2.51	0.16	3.39	0.782
	8	104	121	87	4.17	0.15	5.01	-
Diet 3	9	205	57	89	8.45	0.20	4.62	-
	10	140	21	87	4.33	0.17	3.69	1.162
	11	201	81	99	9.03	0.15	3.97	0.586

TABLE 42. EFFECT OF ENERGY-PROTEIN ON BONE METACARPAL MINERAL CONCENTRATIONS IN SHEEP

EXPERIMENT I, TRIAL 2

	Wether No.	BONE DM FAT FREE Ca %	BONE DM FAT FREE P %	BONE DM FAT FREE Mg %	Ash %	ASH Ca %	ASH P %	ASH Mg %	Spec. Grav. gm/cc	BONE DM FAT FREE Ca mg/cc	BONE DM FAT FREE P mg/cc	BONE DM FAT FREE Mg mg/cc	Ca:P ratio
Diet 1	1	23.0	13.7	0.55	68.3	34.2	19.6	0.74	1.88	4.50	247	9.7	1.75
	2	23.0	13.7	0.52	68.3	33.7	20.0	0.81	1.80	414	346	9.9	1.69
	3	23.4	13.1	0.44	68.9	34.0	19.0	0.64	1.85	434	243	8.2	1.79
	4	22.9	13.9	0.54	68.8	33.2	20.0	0.78	1.85	424	258	9.9	1.65
	5	22.5	13.3	0.46	68.7	32.8	19.4	0.66	1.81	407	240	8.3	1.70
	6	21.1	12.8	0.44	65.4	32.2	19.6	0.67	1.55	326	198	6.8	1.65
Diet 3	7	22.8	13.6	0.60	66.5	34.2	20.4	0.90	1.71	388	232	10.3	1.67
	8	20.7	12.0	0.58	63.8	32.5	18.9	0.90	1.53	318	185	8.9	1.72
	9	24.4	14.4	0.58	68.5	35.7	21.1	0.85	1.81	443	261	10.6	1.70
	10	23.4	13.9	0.46	69.0	33.9	20.1	0.66	1.87	436	259	8.5	1.68
	11	22.2	13.1	0.45	68.9	32.2	18.9	0.66	1.79	397	233	8.1	1.69

TABLE 43. EFFECT OF ENERGY-PROTEIN ON BONE RIB MINERAL CONCENTRATION IN SHEEP

EXPERIMENT I, TRIALS 1 AND 2

		BONE (DM FAT FREE)				ASH				BONE (DM FAT FREE)			
	Wether No	Ca %	P %	Mg %	Ash %	Ca %	P %	Mg %	Sp. Gr. gm/cc	Ca mg/cc	P mg/cc	Mg mg/cc	Ca:P ratio
Trial 1	1	20.9	6.1	0.55	61.3	34.1	10.0	0.89	1.67	348	102	9.1	3.41
	2	19.9	6.0	0.52	59.2	33.6	10.1	0.89	1.72	342	103	9.0	3.32
	3	20.8	11.5	0.53	61.5	33.8	18.7	0.86	1.47	306	169	7.8	1.81
Diet 2	4	19.9	6.7	0.66	61.3	32.5	11.0	1.08	1.59	317	107	10.6	2.96
	5	19.0	10.4	0.63	60.2	31.5	17.3	1.04	1.48	281	155	9.3	1.81
	6	21.8	11.7	0.63	60.4	36.1	19.4	1.03	1.51	329	176	9.4	1.87
	7	19.2	10.8	0.82	59.7	32.2	18.2	1.37	1.53	294	166	12.6	1.77
	8	19.4	6.2	0.60	61.3	31.6	10.1	0.97	1.48	285	91	8.8	3.13
Diet 4	9	21.5	11.4	0.78	60.1	35.8	19.0	1.30	148	317	168	11.5	1.89
	10	17.3	5.6	0.65	59.6	29.0	9.3	1.09	1.45	249	80	9.4	3.11
Trial 2	1	20.3	10.8	0.57	62.0	32.8	17.3	0.91	1.43	290	153	8.1	1.90
	2	13.6	7.2	0.44	60.4	22.4	11.9	0.73	1.61	218	115	7.1	1.90
Diet 1	3	20.2	6.4	0.46	61.6	32.7	10.5	0.75	1.42	287	92	6.5	3.12
	4	21.5	11.3	0.58	60.2	35.8	18.8	0.96	1.63	351	184	9.4	1.91
	5	21.2	11.7	1.68	62.5	33.9	18.7	1.08	1.53	323	178	10.3	1.81
	6	20.6	11.1	0.55	59.5	34.6	18.7	0.92	1.53	314	169	8.4	1.86
	7	27.9	15.4	0.99	76.5	36.5	20.1	1.29	1.39	387	214	13.7	1.81
	8	20.6	10.4	0.64	57.6	35.7	18.1	1.11	1.45	299	151	9.3	1.98
Diet 3	9	18.6	10.0	0.61	53.5	34.8	18.7	1.14	1.42	265	142	8.7	1.87
	10	19.4	9.8	0.57	56.0	34.6	17.5	1.01	1.48	286	145	8.4	1.97
	11	20.5	11.7	0.70	54.8	37.6	21.3	1.27	1.23	252	143	8.6	1.76

TABLE 44. EFFECT OF ENERGY-PROTEIN ON WOOL NITROGEN AND MINERAL CONCENTRATIONS IN SHEEP

EXPERIMENT I, TRIAL 2

	MINERAL CONCENTRATION, PPM, DRY MATTER BASIS											
	Wether No.	N %	Ca	P	Mg	Na	K	Fe	Cu	Zn	Mn	Mo	Se
Diet 1	1	14.46	130	148	31	1094	3976	9.98	3.82	940	0.270	0.040	0.297
	2	14.13	145	80	53	699	4718	9.54	3.34	845	0.350	0.049	0.369
	3	14.37	116	76	36	1138	2966	6.05	5.15	918	0.355	0.012	-
	4	14.27	162	157	67	733	3145	9.49	4.86	839	0.368	0.206	0.421
	5	14.30	119	128	28	414	2051	13.21	4.32	631	0.364	0.032	0.475
	6	14.10	162	114	42	697	3812	10.86	4.45	876	0.429	0.164	-
Diet 3	7	13.95	129	170	73	1233	5481	11.46	2.97	1088	0.340	0.075	0.408
	8	14.25	151	183	56	1045	4497	15.91	6.02	1061	0.290	0.043	0.269
	9	14.25	197	166	66	1052	6026	7.61	4.76	780	0.379	0.163	0.419
	10	14.20	150	141	15	1484	5445	10.53	4.92	902	0.230	0.053	0.279
	11	14.31	146	197	72	915	2053	8.50	3.19	1146	0.460	0.049	-

TABLE 45. MEANS,[a] STANDARD DEVIATION, AND COEFFICIENT OF VARIATION OF BODY WEIGHTS IN EWES FED FOUR EXPERIMENTAL DIETS

Period[b]	Means		Standard Deviation	Coefficient of Variation
	Lbs.	Kgs.		%
1	62.70	28.50	9.2	14.60
2	72.00	32.70	8.6	12.00
3	77.40	35.20	10.2	13.20
4	84.90	38.60	10.4	12.20
5	92.20	41.90	11.0	11.90
6	97.50	44.30	11.6	10.90
7	100.70	45.80	12.5	12.40
8	106.00	48.20	13.2	12.40
9	106.50	48.40	14.6	13.70
10	108.80	49.50	15.5	14.20
11	111.60	40.70	15.9	14.30
12	119.40	54.30	17.3	14.50
13	119.10	54.70	17.6	14.80
14	122.30	55.60	18.6	15.20
15	118.20	53.70	20.2	17.10
16	117.40	53.40	20.5	17.40
17	119.90	54.50	21.6	18.00

[a] Means based on 47 observations on body weights (1st, 2nd); 46 (3rd to 5th); 45 (6th to 14th); and 44 (15th to 17th).

[b] Body weights were monthly.

TABLE 46. ANALYSIS OF VARIANCE-MEAN SQUARES FOR BODY WEIGHTS[a] IN EWES FED FOUR EXPERIMENTAL DIETS

	SOURCE OF VARIATION			
Mean Squares	Energy	Mineral	Energy x Mineral	Error
df	1	1	1	1
WT 1	1.26	13.92	19.01	84.20
WT 2	258.91+	3.29	59.20	74.27
WT 3	1849.35***	7.14	44.20	104.03
WT 4	5265.59***	2.60	13.21	107.02
WT 5	5389.10***	2.22	91.21	120.02
WT 6	7435.63***	36.15	126.85	134.60
WT 7	12863.65***	53.81	180.95	155.30
WT 8	15563.42***	138.93	317.20	173.87
WT 9	17543.64***	167.07	478.04	213.22
WT 10	17736.08***	99.11	689.87+	240.16
WT 11	20546.47***	36.90	559.34	213.02
WT 12	17718.14***	96.22	750.93	298.15
WT 13	24173.52***	30.77	958.55+	310.68
WT 14	29522.44***	4.54	1102.43+	344.40
WT 15	32276.45***	68.46	141.43	408.06
WT 16	35631.26***	0.008	84.77	419.16
WT 17	33447.27***	18.27	67.76	464.59

[a] Degrees of freedom for mean squares for body weights, 43 (1st, 2nd), 42 (3rd, 4th, 5th), 41 (6th to 14th), and 40 (15th to 17th).

+ Significant at ($P < .1$).

*** Significant at ($P < .001$).

TABLE 47. MEANS,[a] STANDARD DEVIATION AND COEFFICIENT OF VARIATION OF HEMATOCRIT, HEMOGLOBIN AND SERUM Ca, P, Mg, Na, AND K IN EWES FED FOUR EXPERIMENTAL DIETS

Variable	Hematocrit %	Hb %	Ca mg/100 ml	P mg/100 ml	Mg mg/100 ml	Na µg/ml	K µg/ml
Period 1							
Mean	46.59	11.14	10.69	7.35	2.36	3165	166
SD	6.15	1.32	0.76	1.47	0.24	288	21.68
CV	13.21	11.85	6.47	19.94	10.34	9.11	13.04
Period 2							
Mean	43.93	14.22	10.29	8.91	2.46	3621	233
SD	6.16	2.24	0.96	1.53	0.34	162	17.05
CV	14.03	14.22	9.31	17.18	14.01	4.48	7.30
Period 3							
Mean	40.87	13.13	12.65	9.18	2.68	3555	187
SD	5.11	1.76	1.47	1.68	0.75	267	17.72
CV	10.50	13.38	11.62	18.31	27.95	7.50	9.46
Period 4							
Mean	32.50	11.94	10.06	9.32	2.59	3316	173
SD	6.72	1.71	1.01	1.65	0.34	249	23.72
CV	20.68	14.28	10.08	17.66	13.28	7.51	13.68

[a] Means based on 45 observations in Period 1 and 2, except Hematocrit and Hb with 46 in Period 1; 36 observations in Period 3, except Hematocrit and Hb with 45 and P with 35; and 44 observations in Period 4.

TABLE 48. ANALYSIS OF VARIANCE-MEAN SQUARES FOR HEMATOCRIT, HEMOGLOBIN AND SERUM Ca, P, Mg, Na, AND K IN EWES FED FOUR EXPERIMENTAL DIETS

Source of Variation	df	MEAN SQUARES						
		Hematocrit %	Hb %	Ca mg/100 ml	P mg/100 ml	Mg mg/100 ml	Na µg/ml	K µg/ml
Period 1								
Energy	1	1684.55***	29.86***	45.00***	24.23***	0.87***	1118026.92***	837.24
Mineral	1	0.51	0.51	3.74*	41.74	0.0003	686627.93**	1622.34^{+}
Energy*Mineral	1	70.95	10.35*	2.50*	9.16*	0.100	56079.60	1916.60*
Error	a	37.86	1.74	0.57	2.15	0.06	83114.50	469.99
Period 2								
Energy	1	539.40***	18.68^{+}	7.99**	12.50	0.42^{+}	2455.54	852.10^{+}
Mineral	1	29.71	36.04	2.38	0.36*	0.38^{+}	2204.93	202.66
Energy*Mineral	1	215.82**	4.34	0.64	0.02	0.69*	11226.64	255.95
Error	a	37.99	5.007	0.92	2.34	0.12	26345.25	290.67
Period 3								
Energy	1	132.22*	56.41***	7.60^{+}	7.21	2.33*	140245.34	3010.94**
Mineral	1	63.68	0.11	5.68	5.30	0.15	34410.25	420.25
Energy*Mineral	1	3.05	0.61	4.26	1.59	0.12	5261.46	0.16
Error	a	26.11	3.09	2.16	2.83	0.56	71050.81	313.92
Period 4								
Energy	1	156.56^{+}	48.12***	4.44*	0.03	1.30**	147.28	804.97
Mineral	1	41.77	0.86	4.71*	2.12	0.40^{+}	220712.12^{+}	91.76
Energy*Mineral	1	66.98	0.22	0.19	1.72	1.71***	10636.50	41.56
Error	a	45.18	2.91	1.03	2.71	0.12	62041.89	562.83

[a] Degrees of freedom for error mean squares for hematocrit, Hb, Ca, P, Na, and K, 41 in Periods 1 and 2, except hematocrit and Hb 42 in Period 1; Period 3, Hematocrit and Hb (41), Ca, Na, K (32), P (31), Mg (33); and Period 4 hematocrit, Hb, Ca, P, Mg, Na, and K (40).

TABLE 48 —CONTINUED

+ Significant at (P < .1).

* Significant at (P < .05).

** Significant at (P < .01).

*** Significant at (P < .001).

TABLE 49. ANALYSIS OF VARIANCE-POOLED PERIOD ANALYSIS OF HEMATOCRIT, HEMOGLOBIN AND SERUM Ca, P, Mg, Na AND K IN EWES FED FOUR EXPERIMENTAL DIETS

		Hematocrit		Hemoglobin		Ca		P		Mg		Na		K	
Source	df	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS
Period	3	4827.97	2609.32**	541.46	180.49**	185.114	61.705**	110.981	36.994**	2.719	0.906**	5736345.5	1912115.1**	122581.4	40860.3**
Energy	3	1961.17	1961.17**	146.62	146.62**	0.098	0.098	32.176	32.176**	4.381**	4.381**	485069.2	485069.2	4806.7	4806.7**
Mineral	1	15.00	15.00	5.51	5.51	15.921	15.921**	29.501	29.504**	0.199	0.199	84723.4	84723.4	187.6	187.6
Energy*Mineral	1	3.36	3.36	4.19	4.19	0.572	0.571	0.160	0.160	1.997	1.997**	8393.6	8393.6	771.5	771.5
Ewe (Diet)	11	2522.19	61.52	157.50	3.84	84.128	2.052**	149.380	3.640**	8.712	0.212	2163772.6	527749.4	12369.5	301.7
Period*Energy	3	588.80	196.27**	6.40	2.13	63.226	21.075**	11.153	3.718	0.392	0.131	779278.4	259756.8**	525.0	175.0
Period*Mineral	3	128.96	42.98	33.28	10.10*	0.493	0.164	20.858	6.952*	0.770	0.257	853555.3	284518.4**	2846.9	949.0
Period*Energy* Mineral	3	339.28	113.10**	13.06	4.35	5.486	1.829	10.942	3.647	0.811	0.270	70979.3	23659.8	1328.4	442.8
Error	113[1]	3503.55	28.72	364.01	2.98	87.260	0.772	231.116	2.064	21.868	0.192	7079378.3	62649.4	51376.3	454.7

[1] Degrees of freedom for error for P and Mg are 112, and 114, respectively.

* Significant at ($P < .05$).

** Significant at ($P < .01$).

F1/41 .05 = 4.075

F1/41 .01 = 7.290

TABLE 51. ANALYSIS OF VARIANCE-MEAN SQUARES FOR BONE RIB MINERAL CONCENTRATIONS IN EWES FED FOUR EXPERIMENTAL DIETS

		MEAN SQUARES										
				DRY FAT FREE			ASH			DRY FAT FREE		
Source of Variation	df	Specific Gravity gm/cc	Ash %	Ca	P	Mg	Ca	P	Mg	Ca	P	Mg	Ca:P Ratio
Period 1													
Energy	1	0.097	0.757	0.020	3.343	0.018*	0.511	7.480	0.042*	4853.40	22.96	0.976	0.275
Mineral	1	0.055	0.00031	0.107	6.035	0.006	0.295	16.572	0.016	1917.77	4577.00	8.070	0.421
Energy*Mineral	1	0.232	0.262	0.267	1.183	0.0019	0.351	2.644	0.005	8530.26	1410.16	11.322	0.084
Error	8	0.089	1.183	0.523	3.663	0.003	1.778	9.961	0.007	4482.50	2690.56	3.160+	0.223
Period 2													
Energy	1	1.340***	100.73	4.177	0.014	0.276***	8.421	4.955	1.562***	68544.97***	16305.52**	0.021	0.001
Mineral	1	0.085	288.61*	51.174**	19.595**	0.008	4.138	8.930	0.327*	27744.08*	9294.52*	1.253	0.246
Energy*Mineral	1	0.003	33.26	13.524	2.246	0.001	10.599	0.156	0.021	6086.66	1285.73	1.309	0.076
Error	27	0.034	47.18	6.185	3.786	0.011	4.421	7.493	0.073	3815.70	1524.23	5.964	0.221
Period 3													
Energy	1	0.241***	42.896**	48.351*	18.049+	0.038	66.410+	24.032	0.198+	41451.61***	13854.77**	0.103	0.039
Mineral	1	0.018	15.540*	4.641	3.066	0.043	35.876	2.211	0.078	18.180	1647.14	9.918	0.357
Energy*Mineral	1	0.0008	3.191	14.700	0.829	0.501	29.782	1.083	0.0007	2922.12	130.81	0.122	0.061
Error	31	0.016	3.652	7.832	5.891	0.017	21.256	17.213	0.051	2675.22	1524.77	5.483	0.235
Period 4													
Energy	1	0.498*	5.611	0.061	0.361	0.105**	0.813	0.015	0.344***	20050.03+	6728.00	4.205	0.012
Mineral	1	0.081	16.820	4.805+	3.125	0.043*	1.575	2.258	0.165*	11287.53	5886.13	2.761	0.032
Energy*Mineral	1	0.279	3.920	1.051	2.205	0.003	0.300	10.012	0.015	14749.03	1012.50	7.031	0.219
Error	28	0.106	7.115	1.493	2.816	0.010	2.327	8.588	0.026	5392.86	2672.04	9.926	0.142
Period 5													
Energy	1	0.033	13.671	8.149	2.532	0.026+	8.090	5.601	0.122**	6267.39+	1471.46	1.499	0.055
Mineral	1	0.004	41.041	27.334**	10.767	0.023+	29.498*	13.764	0.023	8165.83*	3376.89	5.567	0.087
Energy*Mineral	1	0.054+	4.810	1.088	0.025	0.002	0.274	0.277	0.012	869.14	708.76	4.545	0.020
Error	28	0.018	22.827	3.308	5.094	0.007	3.923	10.611	0.016	1985.73	1682.35	2.941	0.199

+ Significant at ($P < .1$).

* Significant at ($P < .05$).

** Significant at ($P < .01$).

*** Significant at ($P < .001$).

TABLE 50. MEANS,[a] STANDARD DEVIATION, AND COEFFICIENT OF VARIATION OF BONE RIB MINERAL CONCENTRATIONS IN EWES FED FOUR EXPERIMENTAL DIETS

	Sp. Gr.	Ash	DRY FAT FREE			ASH			DRY FAT FREE			Ca:P
	gm/cc	%	Ca, %	P, %	Mg, %	Ca, %	P, %	Mg, %	Ca, mg/cc	P, mg/cc	Mg, mg/cc	Ratio
Period 1												
Mean	1.73	61.70	21.04	10.35	0.50	34.11	16.79	0.81	364.17	180.67	8.68	2.12
SD	0.30	1.09	0.72	1.91	0.06	1.09	3.16	0.08	66.95	51.87	1.78	0.47
CV	17.22	1.76	3.44	18.49	11.44	3.18	18.79	10.32	18.38	28.71	20.47	22.32
Period 2												
Mean	1.51	55.77	19.35	10.57	0.61	34.74	18.85	1.12	295.16	160.90	9.19	1.90
SD	0.18	6.87	2.49	1.95	0.11	2.10	2.74	0.27	61.77	39.04	2.44	0.47
CV	12.24	12.32	12.85	18.40	17.21	6.05	14.52	24.15	20.93	24.26	25.56	24.72
Period 3												
Mean	1.48	59.91	20.10	10.45	0.67	33.54	17.44	1.12	298.06	155.03	9.74	2.01
SD	0.12	1.91	2.80	2.43	0.13	4.61	4.15	0.23	51.72	39.05	2.34	0.48
CV	8.45	3.19	13.93	23.22	19.70	13.75	23.79	20.11	17.35	25.19	24.03	24.15
Period 4												
Mean	2.01	60.66	20.78	11.08	0.66	34.26	18.30	1.09	418.91	223.94	13.26	1.93
SD	0.33	2.67	1.22	1.68	0.10	1.53	2.93	0.16	73.44	51.69	3.15	0.38
CV	16.18	4.40	5.88	15.14	15.29	4.45	16.01	14.71	17.53	23.08	23.75	19.57
Period 5												
Mean	1.51	60.71	20.83	10.98	0.61	34.31	18.00	1.00	315.88	167.13	9.21	1.98
SD	0.13	4.78	1.84	2.26	0.08	1.98	3.26	0.13	44.56	41.02	1.71	0.45
CV	8.85	7.87	8.81	20.55	13.74	5.77	18.10	12.57	14.11	24.54	18.62	22.58

[a] Means based on 12, 31, 35, 32, and 32 observations on Periods 1, 2, 3, 4, and 5, respectively.

TABLE 52. ANALYSIS OF VARIANCE-POOLED PERIOD ANALYSIS OF RIB BONE MINERAL CONCENTRATIONS IN EWES FED FOUR EXPERIMENTAL DIETS

SOURCE	PERIOD	ENERGY	MINERAL	ENERGY* MINERAL	EWES (DIET)	PERIOD* ENERGY	PERIOD* MINERAL	PERIOD*ENERGY MINERAL	ERROR
df	4	1	1	1	41	4	4	4	81
Specific gravity									
SS	6.342	1.720	0.090	0.060	1.881	0.336	0.099	0.527	3.706
MS	1.586**	1.720**	0.090	0.060	0.046	0.084	0.025	0.132**	0.046
Ash, %									
SS	444.78	112.95	122.230	0.002	915.890	31.24	252.31	32.520	1319.170
MS	111.20**	112.95*	122.230*	0.002	22.340	7.81	63.08**	8.130	16.290
Dry Matter, Fat Free Bone									
Ca, %									
SS	47.07	34.960	62.230	0.490	369.140	7.140	41.90	11.010	180.970
MS	11.77**	34.960	62.230*	0.990	9.003	1.790	10.48**	2.750	2.230
P, %									
SS	9.93	7.110	18.460	1.830	327.160	5.460	38.87	1.500	208.470
MS	2.48	7.110	18.460	1.830**	7.980	0.680	9.72**	0.380	2.570
Mg, %									
SS	0.265	0.381	0.023	0.002	2.918	0.091	0.073	0.056	0.428
MS	0.066**	0.381**	0.023	0.002	0.022**	0.023**	0.018**	0.014*	0.005
Ash									
Ca, %									
SS	2.690	14.174	48.670	2.349	737.818	21.504	11.692	8.442	224.905
MS	0.673	14.174	48.670	2.349	17.996**	5.376	2.923	2.111	2.777
P, %									
SS	19.414	3.006	18.251	2.297	818.218	10.419	52.243	1.828	534.930
MS	4.854	3.006	18.251	2.297	19.967	2.605	13.061	0.457	6.604
Mg, %									
SS	0.917	1.740	0.244	0.030	2.419	0.541	0.308	0.161	2.356
MS	0.229**	1.740**	0.244*	0.030	0.059**	0.135**	0.077*	0.040	0.029
Dry Matter, Fat Free Bone Ca, mg/cc									
SS	309149.900	123500.500	34754.500	3774.200	194914.600	11200.600	22576.600	32337.800	233501.700
MS	77287.5**	123500.5**	34754.5**	3774.200	4754.00*	2800.200	5644.200	8084.50*	2282.700

TABLE 52—CONTINUED

SOURCE	PERIOD	ENERGY	MINERAL	ENERGY* MINERAL	EWES (DIET)	PERIOD* ENERGY	PERIOD* MINERAL	PERIOD*ENERGY MINERAL	ERROR
P, mg/cc									
SS	93441.100	31809.500	11682.100	6.480	116292.100	4680.600	16498.800	8970.100	115577.500
MS	23360.3**	31809.5**	11682.10*	6.480	2836.200	1170.200	4124.70*	2242.500	1426.900
Mg, mg/cc									
SS	322.913	2.553	0.005	0.293	418.597	7.109	15.592	51.185	297.946
MS	80.728**	2.553	0.005	0.293	10.210**	1.777	3.898	12.796**	3.678
Ca:P ratio									
SS	0.489	0.015	0.115	0.133	13.518	0.236	1.057	0.098	11.096
MS	0.122	0.015	0.115	0.133	0.330**	0.059	0.264	0.025	0.137

* Significant at ($P < .05$).

** Significant at ($P < .01$).

F1/41 .05 = 4.705

F1/41 .01 = 7.209

TABLE 53. MEANS,[a] STANDARD DEVIATION AND COEFFICIENT OF VARIATION OF WOOL N, Ca, P, Mg, Na AND K CONCENTRATIONS IN EWES FED FOUR EXPERIMENTAL DIETS

Variable	N, %	Ca, µg/g	P, µg/g	Mg, µg/g	Na, µg/g	K, µg/g
Mean	13.93	205.11	125.59	94.23	1277.25	3746.86
SD	0.45	138.17	41.24	69.97	345.22	1938.22
CV	3.25	67.36	32.83	74.26	27.03	51.73

[a] Means based on 44 observations.

TABLE 54. ANALYSIS OF VARIANCE-MEAN SQUARES FOR WOOL N, Ca, P, Mg, Na AND K CONCENTRATIONS IN EWES FED FOUR EXPERIMENTAL DIETS

Source of Variation	df	MEAN SQUARES					
		N, %	Ca, µg/g	P, µg/g	Mg, µg/g	Na, µg/g	K, µg/g
Energy	1	0.0446	33166.26	946.63	31057.52*	816196.52*	2883007.74
Mineral	1	0.0085	17768.09	57.89	2874.62	96649.15	3602322.97
Energy*Mineral	1	0.0028	4540.16	760.72	2284.42	1109.09	2027659.35
Error	40	0.2051	19090.05	1700.42	4895.31	119175.95	3756711.79

* Significant at ($P < .05$)

TABLE 55. MEANS,[a] STANDARD DEVIATION AND COEFFICIENT OF VARIATION OF MILK AND COLOSTRUM Ca, P, Mg, Na AND K CONCENTRATION IN EWES FED FOUR EXPERIMENTAL DIETS

	MILK					COLOSTRUM				
Variable	Ca g/ 100 ml	P g/ 100 ml	Mg g/ 100 ml	Na µg/ml	K µg/ml	Ca g/ 100 ml	P g/ 100 ml	Mg g/ 100 ml	Na µg/ml	K µg/ml
Mean	1.086	0.805	0.120	3122.59	7275.71	0.547	0.666	0.181	1771.29	4558.86
SD	0.274	0.263	0.015	1196.68	1966.01	0.235	0.268	0.351	1515.43	2254.60
CV	25.248	32.683	12.882	38.32	27.02	42.928	40.198	193.31	85.56	49.46

[a] Means based on 17 and 21 observations for Ca, P, Mg, Na and K in milk and calostrum, respectively.

TABLE 56. ANALYSIS OF VARIANCE-MEAN SQUARES FOR MILK AND COLOSTRUM Ca, P, Mg, Na AND K CONCENTRATION IN EWES FED FOUR EXPERIMENTAL DIETS

Source of Variation	df	MILK MEAN SQUARES					COLOSTRUM MEAN SQUARES				
		Ca g/100 ml	P g/100 ml	Mg g/100 ml	Na µg/ml	K µg/ml	Ca g/100 ml	P g/100 ml	Mg g/100 ml	Na µg/ml	K µg/ml
Energy	1	0.1200	0.1407	0.00029	2425263.2	846123.2	0.0030	0.000097	0.3283	3294001.9	272332.9
Mineral	1	0.388	0.0005	0.00145*	468605.1	20487.2	0.0454	0.04488	0.0071	3994235.7	8975793.3
Energy*Mineral	1	0.312	0.0872	0.00192*	443437.8	120612.9	0.0728	0.00089	0.0071	3409567.2	340604.8
Error	a	0.075	0.0692	0.00024	1432036.2	3865187.9	0.0552	0.07174	0.1230	2296528.5	5083242.4

[a] Degrees of freedom for error of Ca, P, Mg, Na and K in milk are 13, and 17 for colostrum.

* Significant at ($P < .05$).

TABLE 57. MEANS, STANDARD DEVIATION, AND COEFFICIENT OF VARIATION OF BODY WEIGHTS, HEMATOCRIT, HEMOGLOBIN, AND SERUM Ca, P, Mg, Na, AND K IN LAMBS FROM EWES FED FOUR EXPERIMENTAL DIETS

VARIABLE	N	MEAN	SD	C.V.
Wt.[1] (kg)	28	3.41	1.7179	22.9054
Wt.[2] (kg)	17	12.97	10.4041	36.4678
Hematocrit,[1] %	27	44.5926	8.0301	18.0078
Hematocrit,[2] %	17	32.5000	4.3217	13.2975
Hemoglobin,[1] %	27	14.6778	2.5368	17.2835
Hemoglobin,[2] %	17	11.3118	1.7604	15.5628
Ca,[1] mg/100 ml	22	14.1591	1.0533	7.4388
Ca,[2] mg/100 ml	17	12.8000	3.5982	28.1111
P,[1] mg/100 ml	22	8.5409	1.4389	16.8472
P,[2] mg/100 ml	17	8.6235	1.3912	16.1329
Mg,[1] mg/100 ml	22	2.5036	0.3938	15.7303
Mg,[2] mg/100 ml	17	2.7553	1.0437	37.8815
Na,[1] µg/ml	22	3479.2730	204.3290	4.8728
Na,[2] µg/ml	17	3149.0600	358.0200	11.3691
K,[1] µg/ml	22	175.9550	23.8757	13.5693
K,[2] µg/ml	17	202.5880	16.5457	8.1680

[1] Body weight (kg) and sampling at birth.

[2] Body weight (kg) and sampling at weaning (approximately 60 days).

TABLE 58. ANALYSIS OF VARIANCE-MEAN SQUARES FOR BODY WEIGHTS, HEMATOCRIT, AND HEMOGLOBIN IN LAMBS FROM EWES FED FOUR EXPERIMENTAL DIETS

Source of Variation	df	MEAN SQUARES					
		BODY WEIGHTS (KG)		HEMATOCRIT (%)		HEMOGLOBIN (%)	
		1[1]	2[2]	1[1]	2[2]	1[1]	2[2]
Sex	1	1.6074	57.3362	0.4074	19.1076	1.0818	5.9974
Energy	1	1.5023	11.4720	110.9523	21.5862	6.8587	0.0102
Sex*Energy	1	2.9604	18.6490	90.0698	1.3800	21.0594	0.6546
Minerals	1	0.4289	377.8692	24.8030	3.2894	9.3819	0.7268
Sex*Minerals	1	0.0028	151.6744	54.1445	11.5264	10.9543	1.2252
Energy*Minerals	1	0.0419	22.6370	34.0096	7.1317	0.3054	1.9858
Sex*Energy*Minerals	1	7.9956	60.1510	629.0173**	126.3338*	58.4285**	10.7038[+]
Error	a	2.9512	108.2444	64.4831	18.6769	6.4355	

[1] Body weights and sampling at birth.

[2] Body weights and sampling at weaning.

[a] Degrees of freedom for mean square errors are the following: lambs weight 1, 20; lambs weight 2, 9; hematocrit 1, 19; hematocrit 2, 9; hemoglobin 1, 19; hemoglobin 2, 9.

[+] Significant at ($P < .1$).

TABLE 58—CONTINUED

* Significant at (P < .05).

** Significant at (P < .01).

TABLE 59. ANALYSIS OF VARIANCE-MEAN SQUARES FOR SERUM Ca, P, Mg, Na AND K IN LAMBS FROM EWES FED FOUR EXPERIMENTAL DIETS

		MEAN SQUARES									
		Ca, mg/100 ml		P, mg/100 ml		Mg, mg/100 ml		Na, µg/ml		K, µg/ml	
Variation	df	1	2	1	2	1	2	1	2	1	2
Sex	1	1.3285	1.1359	5.4316	3.2653	0.03788	0.3020	22908.70	241774.21	10.0698	538.831
Energy	1	0.3158	2.2030	3.0037	0.0634	0.9466	0.2444	345.44	145591.60	7.9559	197.253
Sex*Energy	1	1.4774	0.0058	42.7046	0.1231	0.7753	0.2324	54416.02	126070.20	12.1443	351.873
Minerals	1	6.4282	6.3635	19.8789	0.0934	1.1487	0.7641	39774.49	62315.40	87.8648	41.329
Sex*Minerals	1	24.7709	8.9620	16.3840	0.2574	2.1696	0.7341	2094.46	12395.37	110.7563	1.167
Energy*Minerals	1	0.0067**	4.3892	10.0762	1.1912	0.0022	0.1562	5838.51	29272.32	194.9132	665.211
Sex*Energy* Minerals	1	11.1432	12.8747	34.4880***	0.0572	2.0756**	0.0642	52774.30	432.67	4.9162	184.011
Error	a	1.1094	12.9472	2.07044	1.9355	0.1551	1.0894	41750.34	128179.07	570.0512	273.819

[1] Sampling at birth.

[2] Sampling at weaning.

[a] Degrees of freedom for mean squares errors are the following: Ca 1, 14; Ca 2, 9; P1, 14; P2, 9; Mg 1, 14; Mg 2, 9; Na 1, 14; Na 2, 9; K 2, 9.

* Significant at ($P < .05$).

** Significant at ($P < .01$).

*** Significant at ($P < .001$).

TABLE 60. ANALYSIS OF VARIANCE-MEAN SQUARES FOR BONE TAIL MINERAL CONCENTRATIONS IN NEWBORN LAMBS

Source of Variation	df	MEAN SQUARES										
		Sp. Gr.	Ash	DRY FAT FREE			ASH			DRY FAT FREE			Ca:P
		gm/cc	%	Ca, %	P, %	Mg, %	Ca, %	P, %	Mg, %	Ca, mg/cc	P, mg/cc	Mg, mg/cc	Ratio
Sex	1	0.0054	0.4974	12.2535	4.9896	0.2942	167.660	63.654	5.410+	1290.49	770.69	10.378	0.2890
Energy	1	0.0724	51.4387+	40.0751	11.8782	0.4496+	209.381	83.822	4.684+	10294.30+	2435.31	120.219	0.0065
Sex*Energy	1	0.0036	4.0356	9.0153	6.2700	0.0075	69.096	77.832	0.0005	1099.84	1004.01	63.949	0.9423
Minerals	1	0.1033	0.7835	0.0150	0.6048	0.0491	2.536	9.650	1.331	893.62	266.30	193.011+	0.2051
Sex* Minerals	1	0.0001	0.0008	15.0268	9.7332	0.2321	158.886	123.226	4.261	2162.57	1530.66	98.060	0.7592
Energy* Minerals	1	0.1048	22.2531	20.9013	0.2783	0.0876	168.683	0.521	1.446+	6940.72	201.31	3.694	2.1709*
Sex*Energy* Minerals	1	0.0096	14.0941	36.1788	9.8333	0.00013	838.304+	189.330	0.189	4156.36	1386.00	11.425	0.0313
Error	10	0.0429	13.5509	17.7319	6.7074	0.0928	195.834	85.677	1.259	2301.69	835.69	56.889	0.3194

+ Significant at ($P < .1$).

* Significant at ($P < .05$).

TABLE 61. MEANS, STANDARD DEVIATION, AND COEFFICIENT OF VARIATION OF BONE TAIL MINERAL CONCENTRATIONS IN LAMBS FROM EWES FED FOUR EXPERIMENTAL DIETS

VARIABLE		MEAN	STANDARD DEV.	CV
Specific gravity, gm/cc	1	1.2694	0.2070	16.3074
	2	1.2144	0.0737	6.0686
Ash, %	1	23.3333	3.6812	15.7764
	2	45.3556	2.2840	5.0358
Dry Fat Free				
Ca, %	1	8.8333	4.2109	47.6708
	2	15.2111	1.0055	6.6103
P, %	1	3.6167	2.5899	71.6090
	2	8.2333	2.0138	24.4589
Mg, %	1	0.4011	0.3047	75,9558
	2	0.4200	0.0432	10.2814
Ca, % Ash	1	37.2222	13.9941	37.5961
	2	33.5333	1.3846	4.1290
P, % Ash	1	15.1722	9.2562	61.0074
	2	18.1218	4.1708	23.0078
Mg, % Ash	1	1.6372	1.1218	68.5213
	2	0.9250	0.0776	8.3870
Dry Fat Free				
Ca, mg/cc	1	111.8889	47.9759	42.8782
	2	184.1111	16.2007	8.7994
P, mg/cc	1	45.2778	28.9082	63.8464
	2	99.8333	26.1941	26.2378
Mg, mg/cc	1	10.7389	7.5425	70.2352
	2	5.0856	0.5935	11.6710
Ca:P ratio	1	2.6667	0.5651	21.1922
	2	1.8372	0.5223	28.4312

[1] Sampling at birth.

[2] Sampling at weaning.

TABLE 62. ANALYSIS OF VARIANCE-MEAN SQUARES FOR BONE TAIL MINERAL CONCENTRATIONS IN WEANING LAMBS

Source of Variation	df	MEAN SQUARES										
		Sp. Gr.	Ash	DRY FAT FREE			ASH			DRY FAT FREE			Ca:P
		gm/cc	%	Ca, %	P, %	Mg, %	Ca, %	P, %	Mg, %	Ca, mg/cc	P, mg/cc	Mg, mg/cc	Ratio
Sex	1	0.0134	19.4438+	4.0360+	1.5304	0.000186	0.8768	0.7392	0.002876	56.1160	40.7609	0.0661	0.1922
Energy	1	0.0020	12.3251	0.9167	1.1030	0.003320	0.6107	1.2574	0.004141	21.6964	0.1074	0.3846	0.0372
Sex*Energy	1	0.0053	6.2795	0.3995	1.0592	0.000006	0.4332	0.2294	0.002134	4.8329	7.7687	0.0492	0.844
Minerals	1	0.0090	26.8236	1.5926	0.9397	0.002277	1.1784	0.024	0.0000006	0.6560	12.4062	0.0423	0.0164
Sex* Minerals	1	0.0019	1.4078	0.0081	0.4209	0.000052	1.5288	0.8003	0.002852	39.6988	30.5493	0.0643	0.00004
Energy* Minerals	1	0.0014	1.2876	0.1492	0.5729	0.00030	3.3836	5.3319	0.000002	83.8392	121.8516	0.0064	0.0050
Sex*Energy* Minerals	1	0.0075	5.6348	0.4768	0.3473	0.09166	0.1046	0.0318	0.01580	14.3838	0.5938	0.5985	0.1132
Error	10	0.0054	5.2166	1.0110	4.0553	0.001865	1.9171	17.3955	0.00602	262.4633	686.130	0.3523	0.2728

+ Significant at ($P < .1$).

TABLE 63. MEANS,[a] STANDARD DEVIATION AND COEFFICIENT OF VARIATION OF SERUM Fe, Cu, Zn, AND Se IN EWES FED FOUR EXPERIMENTAL DIETS

Variable	Fe µg/ml	Cu µg/ml	Zn µg/ml	Se µg/ml
Period 1				
Mean	1.34	0.60	1.46	
SD	0.36	0.16	0.38	
CV	27.05	26.09	26.01	
Period 2				
Mean	1.71	0.52	1.41	
SD	0.52	0.22	0.50	
CV	30.79	41.63	35.12	
Period 3				
Mean	1.87	0.55	1.38	
SD	0.28	0.14	0.36	
CV	15.10	25.31	26.13	
Period 4				
Mean	2.25	0.35	0.92	0.15
SD	0.84	0.23	0.15	0.05
CV	37.09	66.93	16.57	33.66

[a] Means based on 45 observations in period 1 and 2, 36 and 44 on period 3 and 4, respectively.

TABLE 64. ANALYSIS OF VARIANCE-MEAN SQUARES FOR SERUM Fe, Cu, Zn, AND Se IN EWES FED FOUR EXPERIMENTAL DIETS

Source of Variation	df	MEAN SQUARES Fe µg/ml	Cu µg/ml	Zn µg/ml	Se µg/ml
Period 1					
Energy	1	3.63***	0.08+	0.714*	
Mineral	1	0.84	0.06	0.137	
Energy*Mineral	1	0.60*	0.02	0.014	
Error	a	0.13	0.024	0.14	
Period 2					
Energy	1	0.208	0.212*	2.409**	
Mineral	1	0.0008	0.105	0.114	
Energy*Mineral	1	0.014	0.099	0.081	
Error	a	0.276	0.047	0.25	
Period 3					
Energy	1	1.34***	0.014	1.14**	
Mineral	1	0.0001	0.16**	0.06	
Energy*Mineral	1	0.58	0.004	0.012	
Error	a	0.08	0.020	0.13	
Period 4					
Energy	1	5.80**	0.29*	0.30***	0.0019
Mineral	1	1.66	0.009	0.005	0.00017
Energy*Mineral	1	1.79	0.12	0.06	0.0013
Error	a	0.70	0.05	0.02	0.0024

+ Significant at P (< .1).

* Significant at P (< .05).

** Significant at P (< .01)

TABLE 64—CONTINUED

*** Significant at P (< .001).

[a] Degrees of freedom for error mean square for Fe, Cu, and Zn (41) in period 1 and 2; period 3, Fe, Cu (32) and Zn (34); and period 4, Fe, Cu, Zn (40), and Se (37).

TABLE 65. ANALYSIS OF VARIANCE-POOLED PERIOD ANALYSIS OF SERUM Fe, Cu AND Zn IN EWES FED FOUR EXPERIMENTAL DIETS

		Fe		Cu		Zn	
Source	df	SS	MS	SS	MS	SS	MS
Period	3	19.197	6.399**	1.656	0.552**	8.250	2.750**
Energy	1	8.842	8.842**	0.027	0.027	4.024	4.024**
Mineral	1	1.247	1.247	0.271	0.271	0.074	0.074
Energy*Mineral	1	2.215	0.201	0.201	0.201	0.025	0.025
Ewe (Diet)	41	20.244	0.494	4.231	0.103**	15.189	0.370**
Period*Energy	3	2.261	0.754**	0.536	0.179**	0.597	0.199**
Period* Mineral	3	1.170	0.390*	0.080	0.027	0.083	0.028
Period*Energy* Mineral	3	0.716	0.239	1.466	0.013	0.102	0.034
Error	[a]	26.953	0.239	1.466	0.013	6.181	0.043

[a] Degrees of freedom for error for Fe, Cu and Zn are 113, 113 and 115, respectively.

* Significant at ($P < .05$).

* Significant at ($P < .01$).

TABLE 66. MEANS,[a] STANDARD DEVIATION AND COEFFICIENT OF VARIATION OF LIVER MINERALS IN EWES FED FOUR EXPERIMENTAL DIETS

Variable	Fe ppm	Cu ppm	Zn ppm	Mn ppm	Co ppm	Mo ppm	Se ppm
Period 1							
Mean	352.57	277.44	238.22	9.46	0.69	3.88	-
SD	266.73	179.64	165.07	3.96	0.34	1.57	-
CV	75.61	64.75	69.29	41.87	48.70	40.44	-
Period 2							
Mean	308.05	88.71	146.00	7.37	0.39	5.53	1.27
SD	120.32	118.84	114.23	1.89	0.10	2.43	0.55
CV	39.06	133.95	78.24	25.59	24.94	43.96	43.60
Period 3							
Mean	371.32	151.50	126.73	8.81	4.58	3.99	
SD	171.99	253.90	86.96	3.70	3.83	1.75	
CV	46.32	167.59	68.62	42.02	83.62	43.98	
Period 4							
Mean	261.33	93.67	162.33	7.95	0.64	3.25	0.97
SD	127.61	141.53	129.32	2.05	0.39	0.89	0.47
CV	46.32	151.10	79.66	25.80	61.34	27.40	48.99
Period 5							
Mean							1.80
SD							0.61
CV							33.62

[a] Means based on 9 observations on period 1 and 4, except Se with 11 in period 4; and 21 and 22 on period 2 and 3, except Se with 9 on period 2; and 27 observations on period 5 for Se only.

TABLE 67. ANALYSIS OF VARIANCE-MEAN SQUARES OF LIVER MINERAL CONCENTRATIONS IN EWES FED FOUR EXPERIMENTAL DIETS

Source of Variation	df	MEAN SQUARES Fe ppm	Cu ppm	Zn ppm	Mn ppm	Co ppm	Mo ppm	Se ppm
Period 1								
Energy	1	25166.40	37990.37	8064.65	3.14	0.00004	1.87	
Mineral	1	2702.00	9755.57	22422.00	26.19	0.031	2.46	
Energy*Mineral	1	99141.88	30.68	29781.88	4.99	0.223	0.02	
Error	5	71145.13	32271.70	27249.13	15.67	0.113	2.46	
Period 2								
Energy	1	137125.64**	39486.18	43943.06[+]	5.86	0.041*	14.30	3.92*
Mineral	1	37205.06	18373.57	4866.83	0.51	0.174***	86.76**	0.41
Energy*Mineral	1	7350.00	40633.37	3217.54	9.70	0.0002	0.53	3.90*
Error	a	14476.50	14122.06	13048.45	3.56	0.009	5.92	0.31
Period 3								
Energy	1	5690.31	103865.16	3776.43	60.88*	0.26	0.44	
Mineral	1	24936.89	100013.32	423.94	23.89	28.29	47.08***	
Energy*Minearl	1	127505.21*	740254.01**	10465.00	213.95***	64.12*	56.70***	
Error	18	29579.98	64463.76	7561.52	13.70	14.68	3.07	
Period 4								
Energy	1	7068.80	64.80	1748.45	4.64	0.04	2.49	0.182
Mineral	1	2520.50	31375.13	8385.13	0.39	0.36	0.58	0.008
Energy*Mineral	1	0.00	0.00	0.00	0.00	0.00	0.00	0.037
Error	a	16283.50	20029.79	16722.96	4.21	0.15	0.79	0.224
Period 5								
Energy	1							1.224[+]
Mineral	1							0.041
Energy*Mineral	1							0.299
Error	23							0.368

[+] Significant at (P < .1).

* Significant at (P < .05).

** Significant at (P < .01).

*** Significant at (P < .001).

[a] Degrees of freedom of error for Fe, Cu, Zn, Mn, Co, and Mo in period 2 and 3 are 17 and 6, except Se with 5 and 7.

TABLE 68. ANALYSIS OF VARIANCE-POOLED PERIOD ANALYSIS OF LIVER Fe, Cu, Zn, Mn, Co, AND Mo IN EWES FED FOUR EXPERIMENTAL DIETS

		Fe, ppm		Cu, ppm		Zn, ppm		Mn, ppm		Co, ppm		Mo, ppm	
Source	df	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS	SS	MS
Period	3	43366.8	44720.5	44720.5	14906.8	85439.2	28479.7	38.7	12.9**	148.0	49.30**	36.80	12.27**
Energy	1	134824.2	134824.2*	102.8	102.8	47410.0	47410.1	41.5	41.5	0.017	0.017	3.34	3.34
Mineral	1	44407.3	44407.3	6030.8	6030.8	29.7	29.7	30.7	30.7	10.5	10.5	111.17	111.17**
Energy*Mineral	1	85556.7	85556.7	424616.2	424616.2*	30437.8	30437.8	139.2	139.2**	24.9	24.9**	16.49	16.49**
Ewes (Diet)	32	835523.5	26110.1	1527396.6	47731.1**	412373.6	12886.7	371.9	11.6**	186.2	5.80	125.90	3.93
Period*Energy	3	22541.6	7153.9	199151.2	66483.7**	23936.7	7978.9	20.3	6.8	3.5	1.17	6.86	2.29
Period*Mineral	3	39285.1	13095.0	312879.7	104293.2**	49496.1	16498.9	1.4	0.5	40.0	13.30	51.14	17.04**
Period*Energy*Mineral	2	138617.7	69308.9	504581.0	252290.5**	502.1	251.1	18.7	9.4	25.6	12.90	14.67	7.34
Error	14	396443.4	28317.4	154563.5	11040.2	182141.0	13010.1	38.8	2.8	79.7	5.70	47.10	3.36

* Significant at ($P < .05$).

** Significant at ($P < .01$).

TABLE 69. MEANS,[a] STANDARD DEVIATION AND COEFFICIENT OF VARIATION OF WOOL Fe, Cu, Zn, Mn, Mo AND Se CONCENTRATIONS IN EWES FED FOUR EXPERIMENTAL DIETS

Variable	Fe, µg/g	Cu, µg/g	Zn, µg/g	Mn, µg/g	Mo, µg/g	Se, µg/g
Mean	13.71	3.62	947.70	0.57	0.052	0.457
SD	14.46	1.09	130.04	0.37	0.035	0.244
CV	105.48	30.12	13.72	64.65	67.98	53.44

[a] Means based on 44 observations for Fe, Cu, Zn and Mn; and 43 and 20 for Mo and Se, respectively.

TABLE 70. ANALYSIS OF VARIANCE-MEAN SQUARES FOR WOOL Fe, Cu, Zn, Mn, Mo AND Se CONCENTRATIONS IN EWES FED FOUR EXPERIMENTAL DIETS

Source of Variation	df	MEAN SQUARES					
		Fe, µg/g	Cu, µg/g	Zn, µg/g	Mn, µg/g	Mo, µg/g	Se, µg/g
Energy	1	39.14	0.015	135640.90**	0.00070	0.0014	0.029
Mineral	1	334.71	2.659	23.90	0.00056	0.0023	0.012
Energy*Mineral	1	878.81*	0.0003	3426.90	0.2229	0.0004	0.060
Error	40	209.05	1.186	16909.79	0.1366	0.0012	0.060

* Significant at ($P < .05$).

** Significant at ($P < .01$).

TABLE 71. MEANS,[a] STANDARD DEVIATION AND COEFFICIENT OF VARIATION OF MILK AND COLOSTRUM Fe, Cu, Zn, Mn AND Se CONCENTRATION IN EWES FED FOUR EXPERIMENTAL DIETS

	MILK					COLOSTRUM				
Variable	Fe µg/ml	Cu µg/ml	Zn µg/ml	Mn µg/ml	Se µg/ml	Fe µg/ml	Cu µg/ml	Zn µg/ml	Mn µg/ml	Se µg/ml
Mean	4.892	3.461	41.000	0.760	0.0188	3.224	3.823	65.381	0.513	0.0404
SD	1.287	1.084	37.125	0.271	0.0097	1.301	1.733	29.625	0.278	0.0324
CV	26.303	31.322	90.548	35.598	51.798	40.344	45.318	45.311	54.116	80.233

[a] Means based on 17 and 21 observations for Fe, Cu, Zn, Mn in milk and colostrum, respectively, and Se 13 and 10.

TABLE 72. ANALYSIS OF VARIANCE-MEAN SQUARES FOR MILK AND COLOSTRUM Fe, Cu, Zn, Mn AND Se CONCENTRATION IN EWES FED FOUR EXPERIMENTAL DIETS

Source of Variation	df	MILK MEAN SQUARES					COLOSTRUM MEAN SQUARES				
		Fe µg/ml	Cu µg/ml	Zn µg/ml	Mn µg/ml	Se µg/ml	Fe µg/ml	Cu µg/ml	Zn µg/ml	Mn µg/ml	Se µg/ml
Energy	1	0.538	2.453	293.83	0.3264*	0.000053	0.0553	14.2855*	1255.81	0.0560	0.00048
Mineral	1	0.523	1.349	543.16	0.0043	0.000014	2.9350	0.0055	974.06	0.0735	0.000016
Energy*Mineral	1	2.235	0.080	26.84	0.0647	0.000088	1.6404	1.6544	83.48	0.0013	0.0029
Error	[a]	1.656	1.175	1378.23	0.0732	0.000095	1.6921	3.0021	877.61	0.0772	0.0011

[a] Degrees of freedom for errors for Fe, Cu, Zn, Mn in milk are 13 and Se 9; Fe, Cu, Zn and Mn in Colostrum are 17 and Se 6.

* Significant at ($P < .05$).

TABLE 73. MEANS, STANDARD DEVIATION AND COEFFICIENT OF VARIATION OF SERUM Fe, Cu, Zn AND Se IN LAMBS FROM EWES FED FOUR EXPERIMENTAL DIETS

Variable	No	Mean	SD	CV
Fe[1], µg/ml	24	3.4992	1.5065	43.0537
Fe[2], µg/ml	17	1.7476	0.3626	20.7459
Cu[1], µg/ml	24	0.1975	0.1467	74.2876
Cu[2], µg/ml	17	0.1559	0.1454	93.2451
Zn[1], µg/ml	24	1.4742	0.5139	34.8614
Zn[2], µg/ml	17	0.9412	0.2139	22.7238
Se, µg/ml	13	0.0806	0.0097	12.0045

[1] Sampling at birth.

[2] Sampling at weaning (approximately 60 days).

TABLE 74. ANALYSIS OF VARIANCE-MEAN SQUARES FOR SERUM Fe, Cu, Zn AND Se IN LAMBS FROM EWES FED FOUR EXPERIMENTAL DIETS

Source of Variance	df	IRON		COPPER		ZINC		SELENIUM	
		MS[1]	MS[2]	MS[1]	MS[2]	MS[1]	MS[2]	MS[1]	MS[2]
Sex	1	6.7170	0.0006	0.0263	0.0273	0.1924	0.0296	0.001129	-
Energy	1	0.9525	0.6519	0.0331	0.0124	0.4271	0.0736	0.000010	-
Sex*Energy	1	0.5212	0.2365	0.0228	0.0176	0.7973	0.0119	0.000019	-
Minerals	1	2.0956	0.6705	0.0065	0.0062	0.0105	0.0400	0.000023	-
Sex*Minerals	1	13.0140	0.0038	0.0024	0.0001	0.1065	0.2610*	0.002156	-
Energy*Minerals	1	0.4599	0.0038	0.0013	0.0186	0.0094	0.0140	0.000009	-
Sex*Energy* Minerals	1	8.5127+	1.1642**	0.0007	0.0151	0.5373	0.8080***	-	-
Error	a	2.2696	0.1315	0.0215	0.0211	0.2641	0.0457	0.000094	-

[1] Sampling at birth.

[2] Sampling at weaning.

[a] Degrees of freedom for mean square error are the following: Fe 1, 16; Fe 2, 9; Cu 1, 16; Cu 2, 9; Zn 1, 16; Zn 2, 9; Se 1, 6.

+ Significant at ($P < .1$).

* Significant at ($P < .05$).

** Significant at ($P < .01$).

*** Significant at ($P < .001$).

TABLE 75. EFFECT OF ENERGY AND PROTEIN IN BLOOD METABOLIC PROFILES (SMAC 25) IN WETHERS

	DIET 1						DIET 3				
Wether No.	1	2	3	4	5	6	7	8	9	10	11
Glucose, mg/DL	63	69	65	65	68	69	66	81	62	46	81
Na, meq/L	141	150	146	139	145	136	142	138	140	146	146
Potassium, meq/L	5.3	4.6	5.3	5.3	5.3	4.6	3.8	4.1	3.7	4.6	4.0
Chloride, meq/L	103	106	107	104	108	90	103	102	102	107	106
CO_2 content, meq/L	21	24	25	25	27	23	22	26	19	22	26
Balance = Na - ($Cl+CO_2$), meq/L	17	20	14	10	10	15	17	10	19	17	14
BUN, mg/DL	93	8	6	16	25	27	21	9	14	23	10
Creatine, mg/DL	1.3	1.6	1.2	1.4	1.2	1.0	1.3	1.1	1.3	1.4	1.2
BUN/Creatine ratio	72	5	5	11	21	27	16	8	11	16	8
Uric Acid, mg/DL	0.3	0.3	0.2	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.2
Ca, mg/DL	10.3	9.9	10.9	9.3	9.4	9.0	9.0	9.5	9.7	9.5	8.0
P, mg/DL	4.6	3.5	4.6	6.3	6.9	6.8	6.9	5.5	6.6	7.0	6.9
Total Protein, g/DL	6.4	6.1	6.2	5.5	6.5	6.0	5.8	6.2	5.7	6.2	5.9
Albumin, g/DL	3.3	3.2	3.5	3.0	3.1	3.1	3.3	3.7	3.4	3.5	3.5
Globulin, g/DL	3.1	2.9	2.7	2.5	3.4	2.9	2.5	2.5	2.3	2.7	2.4
A/G ratio	1.0	1.0	1.2	1.2	0.9	1.0	1.3	1.4	1.4	1.2	1.4
Ionized Ca, mg/DL	4.8	4.7	5.1	4.6	4.3	4.3	4.4	4.5	4.2	4.5	4.1
Bilirubin, Total mg/DL	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Alkaline Phosphatase U/L	89	128	261	104	121	86	145	137	123	139	107
LDH, U/L	412	472	363	439	415	371	409	480	447	411	429
SGOT, U/L	69	71	69	67	49	51	74	73	88	59	78
SGPT, U/L	20	9	22	6	8	9	21	9	26	7	14
Cholesterol Total mg/DL	64	76	67	62	63	60	75	41	60	61	54
Triglycerides, mg/DL	29	34	35	15	24	33	50	62	53	83	36
Fe Serum, µg/DL	128	162	154	228	116	93	168	124	149	144	128

TABLE 78. ANALYSIS OF VARIANCE, MEAN SQUARES FOR BLOOD METABOLIC PROFILES (DAY 28) IN EWE LEE FOUR EXPERIMENTAL DIETS

	SOURCE OF VARIATION											
	FIRST PERIOD				SECOND PERIOD				THIRD PERIOD			
Mean Squares	Energy	Mineral	Energy Mineral	Error	Energy	Mineral	Energy Mineral	Error	Energy	Mineral	Energy Mineral	Error
df	1	1	1	41	1	1	1	41	1	1	1	40
Glucose, mg/DL	732.63*	723.56*	5.56	156.68	3934.84***	34.95	45.43	90.57	1769.56*	646.97	144.16	373.90
Na, meq/L	5.53	49.19*	1.04	12.09	18.40+	4.36	6.68	5.43	3.68	11.72	0.85	5.45
K, meq/L	0.75	0.52	0.18	0.53	0.52	0.72	0.0033	0.27	0.26	0.10	0.002	0.27
Chloride, meq/L	66.71**	12.81	4.09	6.33	7.21	10.77	5.17	6.58	21.24	1.01	3.64	9.76
Balance = Na-(Cl+CO_2) meq/L	400.58***	6.31	0.50	9.22	364.75***	1.06	6.67	9.03	307.75***	1.05	42.15	24.57
BUN, mg/DL	2189.35***	0.024	33.96	14.02	1151.60***	24.69	1.08	9.29	832.90***	26.62	1.03	25.93
Creatine, mg/DL	0.08*	0.03	0.07*	0.016	0.11	0.008	0.09	0.045	0.07	0.08	0.013	0.058
BUN/Creatine ratio	1817.19***	2.22	1.89	15.26	488.21***	15.02	9.11	9.83	250.28***	52.79	1.76	20.26
Uric Acid, mg/DL	0.06*	0.04+	0.0033	0.011	0.043+	0.007	0.028	0.012	0.00028	0.00030	0.00033	0.00022
Ca, mg/DL	16.06***	8.33***	2.85*	0.47	14.44***	0.30	0.13	0.81	2.21	0.77	0.000006	1.11
P, mg/DL	94.21***	36.46***	11.10**	1.22	9.50+	0.08	1.08	3.05	7.61	12.85	5.89	8.09
Total Protein, mg/DL	0.007	0.54+	0.13	0.18	6.60***	0.12	0.023	0.17	10.69***	0.03	0.05	0.26
Albumin, g/DL	1.34***	0.21+	0.002	0.062	3.29***	0.004	0.001	0.054	6.80***	0.014	0.027	0.17
Globulin, g/DL	1.15**	0.08	0.10	0.12	0.59*	0.90	0.017	0.087	0.008	1.044	0.32	0.78
A/G ratio	0.52***	0.00007	0.013	0.020	0.33	0.043	0.062	0.12	0.36**	0.014	0.005	0.031
Ionized Ca, mg/DL	3.73***	2.53***	0.57*	0.11	7.05***	0.12	0.06	0.17	3.10***	0.21	0.0011	0.24
Bilirubin, Total, mg/DL	0.011+	0.004	0.0013	0.004	0.0008	0.012	0.000002*	0.0036	0.0009	0.000002	0.0008	0.00046
Alkaline Phosphatase U/L	1878.86	4270.13	569.82	4680.15	47.46	253.69	8443.91	6791.85	6997.10	8467.30	12505.51	6453.69
LDH, U/L	96632.25***	4264.41	10904.14	5996.65	190586.80	3517.00	48724.80	21117.00	34121.10	193943.20	253640.60	115311.20
SGOT, U/L	10299.41***	4429.38	50.30	524.84	1084.60	809.10	2150.70	2263.80	24975.00	60864.20	61087.00	28499.80
SGPT, U/L	167.83	91.93	2.83	204.97	4.45	183.65	1.06	103.15	168.86	720.50	0.071	374.11
Cholesterol, Total, mg/DL	9319.13	3731.08	2846.40	6010.38	90.85	71.51	102.62	151.28	286.90	30.75	1.18	274.96
Triglycerides, mg/DL	45.53	26.41	436.12*	6924.17***	130.35	29.40	281.37+	155.77	95.97	149.40	274.96	197.87
Fe Serum, µg/DL	24905.59***	6654.86*	10758.58**	1243.34	1034.70	1603.50	6413.70+	2004.40	10901.40*	6934.80	50.34	2754.72
CO_2, meq/L	73.98***	0.004	1.28	3.77	306.01***	0.026	5.19	6.90	440.48***	0.55	66.65*	14.04

TABLE 76-CONTINUED

+Significant 4t ($P < .1$).

*Significant at ($P < .05$).

**Significant at ($P < .01$).

***Significant at ($P < .001$).

TABLE 77. ANALYSIS OF VARIANCE, MEAN SQUARES FOR BLOOD METABOLIC PROFILES (SMAC 25) AND SELENIUM IN LAMBS AT WEANING

Mean Squares	SOURCE OF VARIATION Sex	Energy	Sex*Energy	Minerals	Sex*Minerals	Energy*Minerals	Sex*Energy*Minerals	Error
df	1	1	1	1	1	1	1	a
Glucose, mg/DL	3.657	17.424	281.558	120.794	228.167	12.403	-	155.177
Na, meq/L	12.432	0.324	0.019	1.297	12.042	1.470	-	3.990
K, meq/L	0.022	0.096	0.043	0.138	0.0004	0.00003	-	0.075
Cl, meq/L	8.575	3.136	3.769	27.708*	2.042	7.680	-	2.771
CO_2, meq/L	0.090	7.396	37.231*	15.297	12.042	4.081	-	6.396
Balance, Na - ($Cl+CO_2$) meq/L	0.090	2.304	16.173	0.708	2.042	3.203	-	14.490
BUN, mg/DL	40.889+	360.000***	2.769	0.120	63.375*	12.000	-	11.500
Creatine, mg/DL	0.0036	0.0004	0.0048	0.0063	0.0067	0.0003	-	0.0124
BUN/Creatine ratio	35.714	504.100**	12.019	9,422	60.167	10.083	-	22.260
Uric Acid, mg/DL	-	-	-	-	-	-	-	-
Ca, mg/DL	0.0321	0.9986	0.0017	0.0432	0.2400	0.2883	-	0.2916
P, mg/DL	6.665+	0.433	0.012	2.637	2.160	1.153	-	1.736
Total Protein, mg/DL	0.0006	0.2822+	0.2633+	0.0565	0.7350**	0.1587	-	0.0690
Albumin, g/DL	0.0858	0.0922	0.0931	0.0179	0.3504+	0.0021	-	0.0752
Globulin, g/DL	0.0723	0.0518	0.0433	0.1379	0.0704	0.1240	-	0.0593
A/G ratio	0.0543	0.00004	0.0008	0.0796	0.0204	0.0085	-	0.0310
Ionized Ca, mg/DL	0.0173	0.0846	0.0492	0.0432	0.0150	0.0085	-	0.0542
Bilirubin, Total, mg/DL	0.0023	0.0010	0.0031	-	-	-	-	-
Alkaline Phosphatase, U/L	29973.6000	53582.4000	7368.5000	25428.5000	21182.0000	15914.1000	-	8094.0000
LDH, U/L	651.180	749.960	15335.600	185.3500	40.042	1761.760	-	4049.740
SGOT, U/L	5805.800	2073.600	263.250	1588.3000	3978.380	80.084	-	4496.010
SGPT, U/L	752.432*	256.036	808.173*	13.0610	100.042	7.363	-	134.990
Cholesterol Total mg/DL	345.432	222.984	595.692	142.3550	9.375	198.453	-	205.917

TABLE 77—CONTINUED

Mean Squares	SOURCE OF VARIATION							
	Sex	Energy	Sex*Energy	Minerals	Sex*Minerals	Energy*Minerals	Sex*Energy*Minerals	Error
Triglycerides, mg/DL	207.430	278.780	7.692	0.199	828.38^{+}	24.653	-	199.73
Fe Serum, µg/DL	2228.930	5760.000*	651.08	6165.190*	8288.17*	3468.000	-	1078.58
Se, µg/ml	0.00009	0.00026	-	0.00292^{+}	-	-	-	0.00062

[a] Degrees of freedom for mean square errors for SMAC 25 are 8, and Se is 4.

$^{+}$ Significant at ($P < .1$).

* Significant at ($P < .05$).

** Significant at ($P < .01$).

*** Significant at ($P < .001$).

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BIOGRAPHICAL SKETCH

Oswaldo R. Rosero Puga was born in Quito, Ecuador, on April 8, 1942. He received his elementary and secondary education in the public school of Quito, Ecuador, graduating from Juan Pio Montufar High School in 1960. In 1961, he enrolled at Central University, School of Veterinary Medicine, Quito (3 years). In 1964, he attended Central University of Venezuela, School of Veterinary Medicine (Maracay), and was graduated in 1967 with a veterinary medicine degree. In July, 1968, he obtained Venezuelan citizenship. He was an Assistant Professor of Animal Nutrition in the School of Veterinary Medicine of the University of Zulia, Maracaibo, Venezuela, until May of 1973.

In August, 1973, he enrolled in Graduate School at the University of Kentucky, Lexington, Kentucky, U.S.A., and graduated with a Master of Science degree in animal science, majoring in animal nutrition in July, 1975. He went back to the University of Zulia and was an Associate Professor of Animal Nutrition until December, 1978. In January, 1979, the author was enrolled in the graduate school at the University of Kentucky. In June, 1979, he was transferred to the University of Florida to pursue his studies toward the Doctor of Philosophy degree.

He is currently a member of the Latin American Society of Animal Science, the American Society of Animal Science, the American Society

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He is married to the former Yully I. Roa Avila and has three children, Romina, Roberto and Roxana.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

L. R. McDowell

Dr. L. R. McDowell, Chairman
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